SOIL FACTORS AFFECTING BEAN (*Phaseolus vulgaris* L.) AND MAIZE (*Zea mays* L.) PRODUCTION IN HONDURAS, CENTRAL AMERICA

BY

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Bean and maize in Central America are grown in marginal soils. Glasshouse and field experiments were conducted in various soils of the Yeguare Valley, Honduras to determine soil factors which limit biological N_2 fixation (BNF) by beans. Rhizobium leguminosarum biovar. phaseoli exotic and native strains were tested in acid soils with lime corresponding to 0, 0.5, 1, 1.5, and 2.0 times the recommended amounts to increase pH to 6.5. Phosphorus consisted of 0, 10, 20, and 40 mg kg^{-1} and Mo consisted of 0, 0.06, 0.13, 0.25, and 0.5 mg kg^{-1} in glasshouse experiments. In field experiments, two levels each of lime, P, and Mo were tested with inoculation and combined N. Different water regimes were also studied.

Response to Rhizobium strains was cultivar and site specific. A mixture of exotic and native strains was used in subsequent experiments. Plants fertilized with combined N usually produced higher shoot and root dry weight, total plant N, and bean yield than did inoculated plants, but reduced nodule dry weight. Lime applications did not consistently affect the measured parameters. However, pH values in the range of 6.2 to 6.5 were optimum for bean plant and nodule dry weight. For soils containing 6 to 17 mg Mehlich I-extractable P kg^{-1} applied P was linearly correlated with plant and nodule dry weight. Current P (19 mg kg^{-1}) recommendations may underestimate the needs for P fertilization. Responses to Mo were mixed. Water to give close to 2/3 of field capacity was sufficient to obtain yields comparable to those obtained with full field capacity for the entire cycle.

Yield of maize was not affected by timing of application of ammonium sulfate. However, seedling, root and plant dry weights were increased by the application of ammonium sulfate at planting as compared to 15 d after planting. In control treatments without applied N, at planting plants grew slowly through the first 15 d. Application of 25 to 50% of the N fertilizer at planting and the remainder 30 d after plant emergence is a suggested treatment.

This research indicated that the Honduran soils studied are not typical "extremely weathered" tropical soils. A need for lime and Mo was not consistently found. Lack of N,

P, and water were the most important limiting factors for maize and bean production and BNF.

CHAPTER 1
INTRODUCTION

The maize (Zea mays L.)-bean (Phaseolus vulgaris L.) cropping system is one of the most common plant associations in Latin America, especially in Central America. Both crops constitute major sources of carbohydrate and protein for human consumption. In many cases, beans are the major protein source, especially in rural areas where daily human consumption ranges from 54 to 72 g. In urban areas, bean consumption ranges from 47 to 52 g (Bressani, 1981). Vast numbers of indigenous and introduced black-and-red bean cultivars are grown in the region. Consumers in Guatemala, Costa Rica, and part of El Salvador prefer black beans. Red cultivars are preferred in Nicaragua, Honduras, and part of El Salvador. According to CIAT (1979) bean yield can be as high as 4,000 kg ha^{-1}. However, average yields in Central America and Panama are around 600 kg ha^{-1} (FAO, 1984a).

Average yield for maize in Central America is 1,400 kg ha^{-1}; this is less than one half the world average of 2,981 kg ha^{-1} (FAO, 1984a) and only 22% of the average U. S. production. Throughout the region, beans produced by small

farmers are generally relay planted or interplanted with maize or sorghum. In Honduras, between 65,000 and 70,000 ha are devoted to maize-bean production each year (Torres, 1979). Average bean production in Honduras is around 500 kg ha^{-1} (FAO, 1984a; Ramos, 1984). Maize yields average 1,400 kg ha^{-1} in Honduras (FAO, 1984a).

Generally, beans and maize are grown on marginal, hillside soils with low inputs. Low soil fertility conditions are important factors limiting growth of these crops. Farmers understand the value of fertilizer but few use fertilizers due to economic constraints. Some international and national centers are studying more suitable ways to increase yields using low-cost methods and inputs which are easy to handle and available to resource-scarce farmers.

Bean-inoculation technologies may prove to be an economically sound means to obtain biological N_2 fixation (BNF) to meet the bean crop's N requirement. However, beans are generally considered to be weak N_2 fixers and have given variable response to inoculation (Graham and Halliday, 1977; Graham, 1980). Characteristics of both host plant and bacteria, as well as the environment affect BNF.

Due to the importance of the maize-bean cropping system, it was decided to study soil characteristics that could affect yields of these two crops under Honduran conditions. More emphasis was given to the bean crop. Pot

and field experiments were conducted with the following objectives:

a) to determine the value of exotic versus indigenous strains of *Rhizobium leguminosarum* bvar. *phaseoli* for use as bean inoculants in Honduras,

b) to identify major soil factors which limit BNF in beans under Honduran soil conditions,

c) to determine appropriate management practices to correct these limiting factors as a basis for practical recommendations for bean cultivation by Honduran farmers, and

d) to determine the effect of split applications of ammonium sulfate on maize plant growth and yield.

CHAPTER 2
LITERATURE REVIEW

Introduction

In this research emphasis was given to the bean crop due to its potential to increase yield through inoculation, and to the lack of information on the soil factors limiting both BNF and grain yield, especially in field trials. Hence, the literature was reviewed more extensively for bean than for maize.

Biological Nitrogen Fixation

Legume plants can meet their N requirement for growth in two ways: 1) by taking available N from the soil and 2) by reducing atmospheric N_2 through their symbiosis with Rhizobium. The legume-Rhizobium association is one of the most elaborate and most efficient associations between plants and bacteria. It is estimated that 25% of terrestrial N_2 fixation (139×10^6 Mg yr^{-1}) can be accounted for by legume plants (Burns and Hardy cited by Bergersen, 1982). The symbiotic fixed N helps increase yields of legume crops in an economical way, especially in Less Developed Countries

(LDCs) where economic resources are scarce and where legumes play an important role as protein sources. Other important benefits of legumes and their BNF capacity include decreased soil erosion and improved soil fertility; depending on the legume, BNF can range from 50 to more than 100 kg ha^{-1}. Hubbell et al. (1989) cited among other benefits derived from legume inoculation, the fact that inoculated plants contain more N than do noninoculated plants, the quality of the former is superior because more protein means more weight gain for the animals that consume the plants.

Rate of Fixation

Rate of N_2 fixation varies according to the legume, the bacteria, and the environment. Soybean is commonly reported to have a good N_2-fixation rate. On the other hand, beans have been reported to fix variable amounts. Rennie and Kemp (1983a and 1983b) reported that some strains fixed more than 100 kg ha^{-1} with P. vulgaris. They concluded that R. phaseoli was as efficient as other rhizobia such as R. japonicum in supplying the necessary fixed N_2. Furthermore, they claimed, in N_2-fixing mode, that some of the bean cultivars can meet their genetic potential without use of combined N. In most cases, however, the potential for N_2 fixation is substantially higher than the amount actually fixed. The potential quantity depends on characteristics of the plants and the bacteria, as well as their mutual,

genetically controlled compatibility and the influence of the environment on each one and on their interactions (Graham and Rosas, 1977; Freire, 1984). Cultivars and strains differ in their BNF (Franco and Doberenier, 1967; Burton, 1974) and interactions of the host and strains have been reported in most legumes. This makes the selection of cultivars with high fixing potential more difficult (Graham, 1981).

Characteristics of the Symbionts

Host plant

The genus Phaseolus belongs to the family Leguminosae, subfamily Papilionoidae, and tribe Phaseolinae. Bean is the most common species in Central America. Cultivars vary greatly in color, size, shape, and nutritional value.

The host plant plays a major role in symbiotic N fixation. Host genes involved in nodule initiation, development, and function appear to play a more important role in controlling the symbiosis than the Rhizobium strain and the environment. Beans have been proven to be weak N_2 fixers and generally respond to fertilization (Bazan, 1975; Duque et al., 1983; Cuautle, 1979). Graham and Rosas (1977) reported gains of 25 to 30 kg N ha^{-1} during the bean growth cycle; still, the N_2 fixed constitutes less than 50 % of what the plants need (Graham and Halliday, 1977). Jansen and Vitosh (1974) stated that symbiotic fixation does not produce sufficient N for optimum yield under conditions in

Michigan and an application of 44 kg N ha^{-1} is needed to complete the cycle. Rosas and Bliss (1985) calculated a need for 52 kg N ha^{-1} to increase Central American bean yield from 600 to 1,000 kg ha^{-1}. They suggested that this amount could be obtained through BNF.

Genotypes vary in their use of nutrients and in their ability to create a successful association with rhizobia, and fix N_2. Haag et al. (1978) applied two levels of N-P fertilizer to 124 bean genotypes (ranging from primitive to recent breeding lines). They inferred that beans have unique genetic properties that regulate their response to N and P fertilization. The main effect of high fertilization was to enhance the role of pods/plant and single-seed weight that influence seed yield/plant. The response they observed was not specific to improved or unimproved lines.

The behavior of legumes that fix N_2 is different from legumes that depend on mineral N. Symbiotic N_2 fixation requires substantial changes in root morphology and physiology. The root nodules compete with other plant organs for the assimilates they require. A nonnodulated legume does not have this additional sink (Cassman et al., 1980).

Some of the bean plant's characteristics contribute to their poor behavior. Although the majority of legumes exhibit a distinct primary root around which the first nodules are clustered, P. vulgaris has a fibrous root

system, with many small nodules located mostly on secondary roots.

Nitrogen fixation efficiency varies among cultivars of P. vulgaris. Amounts of N_2 fixed varied from 25 kg N ha^{-1} for a 60-d (precocious) cultivar to 65 kg N ha^{-1} for a 90-d cultivar (Ruschel et al., 1982). Graham and Rosas (1977) determined that bush bean fixed less N than the more primitive climbing ones. In their study, bush cultivars assimilated more combined N than climbers in the pre-fixation period; assimilation could have resulted in a depression of nodule activity. Another important characteristic of the host is its reduced period of N_2 fixation in comparison to other leguminous plants. This characteristic might be related to carbohydrate supply and partitioning. Graham and Halliday (1977) found that cultivars with high N_2-fixing capacity were the ones which not only had a higher soluble carbohydrate percentage in all organs but also partitioned more of the total carbohydrates to the nodule. They found a strong correlation between supply of N and acetylene reduction.

Graham and Rosas (1977) determined that recently germinated bush-bean plants absorbed N from the soil more rapidly than climbing beans. They suggested that this could contribute to a decrease in the amount of carbohydrates to the nodules and lower the N_2 fixation.

The effects of plant roots on soil are of great importance to root-bacteria symbiosis. Rhizosphere microflora are dependent on the root environment, and are unique for every plant. They are more affected by the plant than by the soil (Henis, 1986). The rhizosphere population thrives on root exudates, root excretions, and moribund and sloughed-off root tissue. Soil organic matter affects symbiosis indirectly by favoring root growth and exudation and by providing nutrients. The rhizosphere population exerts both favorable and unfavorable effects on plant growth. Unfavorable effects are enhanced denitrification, competition for oxygen, oxidation of Fe and Mn, and support of specific pathogens. Favorable effects include antagonism toward certain pathogens, P solubilization, symbiotic N_2 fixation, and production of chelating agents and growth factors.

Rhizobium

Alexander (1984) listed several characteristics of an ideal Rhizobium strain as one that is able to survive well in the soil, to grow and colonize the roots readily, and to survive in the presence of other organisms that could compete and could be antagonistic. In other words, the ideal strain should survive and reproduce under harsh conditions. It should be both infective (able to nodulate the legume crop), and effective, i.e., able to fix sufficient N_2 to sustain a level of legume production close to or surpassing

the production possible if the legume were supplied with combined N (Halliday, 1984). There are some legumes that never seem to nodulate. Similarly, some strains of Rhizobium are able to cause the formation of the nodule but do not fix N_2; they are ineffective.

Effectiveness and Specificity of the Symbionts

Success in N_2 fixation depends largely on the genetic compatibility between the symbionts. Associations between symbionts are specific (Casas, 1984). For most legumes there are rhizobia that are able to produce N_2 fixing nodules. Most naturally occurring bacteria associate effectively with a particular legume species. There are hosts that are particularly selective in their association while others are more promiscuous. This specificity between the host and the bacteria implies a physiological association between the microsymbiont and the root surface of the host. Several authors have shown correlation between infectivity and lectin-Rhizobium binding (Dazzo and Hubbell, 1975).

Rhizobium leguminosarum biovar. phaseoli is the specific bacteria for beans. This group is characterized by being fast growing, Gram-negative, and rod-shaped.

Rhizobium - Host Plant Symbiosis Limiting Factors

Generally, beans are planted without seed inoculation because responses are often times poor, and good results may

be restricted to a narrow environmental range (Graham and Halliday, 1977; Boucher and Espinosa, 1982). Legume-rhizobium symbiosis is affected by several soil characteristics. The extent to which these affect N_2 fixation is usually site specific and must be considered when evaluating the effectiveness of the symbiosis (Wynne et al., 1987). Soil N, pH, and P have been reported to affect growth, survival, and invasion of rhizobia (Wynne et al., 1987; Robson, 1978). Molybdenum is indispensable to BNF but its excess depresses plant development and to an even greater extent the symbiosis process (Franco and Doberenier, 1967).

Some of the most commonly mentioned soil factors that could potentially affect BNF in tropical environments are soil acidity, and P, and Mo deficiencies. It has been claimed that legumes depending on N_2 fixation need more P and Mo to accomplish their task.

Influence of mineral N and organic matter on BNF

The presence of N may or may not benefit BNF. In some cases, N present in the soil or applied as a fertilizer results in an enhancement of BNF. This is the case with soybean (Glycine max L.) and other species. Freire (1984) mentioned that mineral N in small amounts is beneficial to nodulation. This author claimed that if small amounts of N are not added to beans, nodule formation and N_2 fixation are poor. Mangual-Crespo et al. (1987) found that 22 kg N ha^{-1}

enhanced BNF in beans in Puerto Ricans soils. However, researchers report that application of high amounts of N depresses BNF. Bean seems to be very sensitive to the presence of N. Graham (1981) determined that doses as small as 15 kg mineral N ha^{-1} reduced BNF of beans by 40 %. Presence of high soil N could be one of the possible causes for a low BNF. Combined N close to the roots could affect the nodulation by decreasing the amount of indole acetic acid (IAA) or hastening its destruction as reported by Tanner and Anderson (1964).

Nitrogen may affect this symbiosis during several stages of development. Effects of mineral N depend on the host species, rhizobia strains (Gibson, 1977), source of N, amount applied, time of application (Awonaike et al., 1980), and soil characteristics (Saito et al., 1984).

Evans (1982) reported that establishment of symbiosis involving Rhizobium japonicum strains was highly susceptible to the effects of 200 mg NO_3^--N kg^{-1} of soil but 50 mg NO_3^--N kg^{-1} had little effect. Once symbiosis was established without any N, the response to application of N as nitrate or ammonium varied significantly for the different strains. He found that N-tolerant strains were symbiotically effective.

Continuous application of combined N to nodulated soybeans stimulated vegetative growth and affected the rate of N_2 fixation. Application of combined N induced less

nodule tissue and lower nitrogenase activity per plant inoculated with Rhizobium strains. There was a varied response among the strains; some strains were less sensitive than others. The proportion of the plant N estimated to come from fixation and fertilizer was affected by plant genetics. Nodulated plants had nitrogenase activities comparable with other grain legumes and responded to combined N during vegetative growth, but this was not reflected in grain yield (Awonaike et al., 1980).

Soil organic matter contributes to soil fertility through several mechanisms. Organic matter may improve activity of the soil organisms, as well as supply available N, P, K, and micronutrients at a continuous and steady rate. It also improves soil structure, and produces growth factors and chelating agents which improve the uptake of micronutients (Henis, 1986). Diaz-Romeu et al. (1970) analyzed 167 samples from Ah horizons of Central American soils and reported organic carbon to be between 0.40 and 12.2% C with an average of 2.96% C. More than half of these samples were in the range of 0.5 to 2.0% C.

Organic matter has been reported to increase bean yield, through the availability of nutrients, reduction of toxic elements, and improvement of physical characteristics of the soil as well as the symbiosis. Ruschel and Saito (1977) determined the need of inoculants when organic matter, such as fermented manure and sugar cane husks, was

applied to beans. Waters et al. (1980) found that mulching beans with rice hulls increased fresh weight of nodules, roots, stems, leaves, and total plant by 50, 38, 49, 24, and 38% respectively, but pod and final seed weight were unaffected. Some of the benefits of mulching in their experiment were reduction of soil temperature and decreased loss of soil moisture.

Acidity and Liming

Soil acidity results from prolonged leaching with input of biologically generated acids. The most acidic soils coincide with high rainfall, low relief, free drainage, and old stable land surfaces. The acids responsible are mainly carbonic, sulfuric, and nitric; this last one originates from the oxidation of symbiotically fixed N_2 (Henis, 1986).

In most cases, the adverse effects of acidic soils on legumes and Rhizobium cannot be attributed to the hydrogen ion concentration itself but rather to deficiencies of P, Ca, Mg, or Mo, or to the toxic effects of soluble Al and Mn (Keyser and Munns, 1979; Munns and Franco, 1983; Freire, 1984; Doberenier, 1966). The relative importance of these elements in acidic soils varies from soil to soil. Blue and Dantzman (1976) claimed that the concentration of Al in the soil solution is determined by the amount of exchangeable Al and soil characteristics, such as type of inorganic colloid

and the amount of organic matter which defines the cation-exchange capacity of a soil.

Malavolta (1976) reported the optimum pH for bean growth to be from 5.5 to 6.7. Schwartz and Galvez (1980) reported the optimum pH for beans to be in the range of 6.5 to 7.5, but excellent growth is possible at pH 4.7-5.0. Low soil pH is often associated with Al and Mn toxicity, as well as Ca and P deficiencies. These nutrient deficiencies interfere with legume growth and BNF in several ways. They stop growth of rhizobia and nodule initiation, impair nodule function, and slow plant growth. Nodule initiation has been reported to be delayed in solution culture at pH below 5.4 (Franco and Munns, 1982). Freire (1984) mentioned acidity to be the cause of inhibition of rhizobia survival, colonization, or competitiveness for nodule sites. Munns et al. (1981) claimed that rhizobia growth, root infection, and nodule activity are affected by acidity.

Lowendorf et al. (1981) reported that R. phaseoli was able to grow in culture at pH 4.4 but exhibited increasingly poor survival in sterile soils when pH was lower than 4.4. They concluded that abiotic factors other than soil acidity limit the ability of the bacteria to live.

Doberenier (1966) found that additions of 40 mg Mn kg^{-1} to two acidic soils decreased numbers and weights of nodules, as well as the N content of bean plants. In the absence of Mn toxicity, nodulation and N_2 fixation were

plentiful in a soil with pH 4.4. In plants dependent on BNF, total plant N decreased linearly with increasing concentration of Mn in the plants. This did not happen with plants fertilized with combined N. Reduction of 30 to 60 % of plant dry weight has been reported when 25 mg Mn kg^{-1} was applied (Franco and Doberenier, 1971).

Liming is a common practice in acidic soils. Van Lierop et al. (1982), increased potato yield by 40% when 2,000 kg lime ha^{-1} were applied. Use of lime in legume crops (Sumner, 1979) has been reported to be beneficial (Awan, 1964; Franco, 1977; Haynes, 1982; Bouton et al., 1981; Munns et al., 1977) and detrimental (Munns and Fox, 1976; Haynes and Ludecke, 1981; Kamprath, 1971; Haynes, 1982; Summer, 1979). Benefits of liming include pH changes; Al and Mn neutralization; improved Ca, P, and Mo availability; improved structure; and better water retention. Primary detrimental effects of lime reported include decreased P availability and micronutrient deficiencies (Haynes and Ludecke, 1981). However, Munns and Fox (1976) found that the detrimental effect of lime was strongly species-dependent and in most cases transitory in 23 legume species they studied.

On the other hand, it is commonly believed that legumes are more resistant than other plants to acidity. Munns and Fox (1977), comparing lime requirements of tropical and temperate legumes in a Hawaiian soil, reported a 4-fold bean

yield increase due to lime applications. The average lime required for beans was 9 to 10 Mg ha^{-1} to obtain 90% of the maximum attainable yield.

Phosphorus

Tropical soils are characterized as being P-deficient. Some authors have shown that this element is perhaps the most important limiting nutrient (Graham, 1981; Freire, 1984). In some cases, it is mentioned as the second most important problem after N deficiency. In Central America, Bazan (1975) reported that plants often respond positively to P application. Similar reports have been presented by Fassbender and Bornemiza (1987). Awan (1964), Burgos (1967), and Sierra (1959) have reported improvement in crop yields when P was applied in Zamorano soils.

Legumes that depend on N_2 fixation need more P than plants that use N fertilizers (de Mooy and Pesek, 1966). This is attributed to the nodulation growth on the roots that limit root extension and to the immobility of P in the soil (Cassman et al., 1980). These authors found that the ratio of root to plant dry weight of N-fixing plants was less than for plants supplied with combined N. Phosphorus plays a vital role in energy required for the reduction of N_2 to NH_3. Both host and bacteria show evidence of differences in P requirements (Freire, 1984). Graham and Rosas (1979) found that nodules were a strong sink for P. Nodule weight increased 9-fold, and P concentration in

nodules by almost 50% over the range of P applied. The P supplied was correlated with N_2 fixation, specific nodule activity, and non-structural carbohydrates in the nodules.

One of the important factors relevant to P availability is the function of mycorrhizal infection in the enhancement of plant P uptake. In general, mycorrhiza and added P have similar effects on plant growth, nodulation, and N_2 fixation (Munns and Mosse, 1980). Plant species, fungal endophyte species, and available soil P affect the extent to which mycorrhiza enhance plant P uptake. However, controversial effects of the interactions between N_2 fixation, mycorrhizal colonization, and beans have been reported by Bethelenfalvay et al. (1982). They found that nodule activity measured by acetylene production was higher in the controls than in the treatments where mycorrhizae were applied at different levels of P. They attributed this to the competition of the two microorganisms for the P and photosynthate which results in plant-growth inhibition.

Molybdenum

Positive responses to Mo applied in the field have been obtained by seed treatment or foliar application in soils with low pH and non-toxic amounts of Al or Mn or both (Graham and Morales, 1974). Recommendations for Mo application vary from 0.25 to 1.2 kg Mo ha^{-1}. Lozano de Yunda and Mora de Gonzalez (1983) determined that positive response to Mo varied in two Colombian soils. One soil was

favored by an application of 250 g Mo ha^{-1}; however, the second one needed 750 g Mo ha^{-1}.

Critical levels of Mo in plants are related to N nutrition (Bender and Barros, 1984). Plants dependent on N_2 for growth require Mo mostly in the nodules were nitrogenase is located. Franco and Munns (1981) suggested that the critical Mo concentration in bean nodules is between 3 and 5 mg kg^{-1}. Bellintani and Lam-Sanchez (1974) found a linear effect of Mo on nodule dry weight.

Interactions

N-P

An interaction between N and P in production of soybean dry matter at high levels of P (0.50 to 2.00 μg P mL^{-1} in nutrient solution) was found by Cassman et al. (1980). The presence of 5.0 mM N in the solution was sufficient to inhibit nitrogenase activity at all but the highest P level indicating that N was not enough to inhibit nitrogenase activity (in 5-wk-old plants) in the presence of high amounts of P (2.00 μg P mL^{-1}) in the nutrient solution.

Nutrient-use efficiency, especially for macronutrients other than N, might also be affected by the way legume plants obtain their N.

Lime-Mo

Three experiments conducted on a Spodosol showed that soybean yields were affected more by pH than by Mo fertilization rate (Forbes et al., 1986). Higher yields were

obtained when lime was applied. They found small, non-significant increases in yield when Mo was applied to limed plots, but not to the unlimed plots.

Highly weathered soils are reported to be Mo deficient. Considerable information concerning Mo deficiency comes from Brazil, where Oxisols show deficiencies of this nutrient. Franco (1977) reported a 93% yield increase when 0.5 kg Mo ha^{-1} and 4 Mg lime ha^{-1} were applied.

Lime-P

Awan (1964) found a positive relationship between lime and P on Zamorano soils for bean, maize, and sorghum (*Sorghum bicolor* L.) cultivars. However, lime has also been reported to cause P deficiency by Summer (1979) and Haynes (1982). According to Haynes (1982) plant uptake of P in response to lime in highly weathered soils is often controlled by two opposite effects. First, lime alleviates Al toxicity bringing about more efficient use of available P. Second, the precipitation of Al as polymeric hydroxy-Al cations following lime application creates new, highly active phosphate adsorbing surfaces in the soils; in this case the potentially available P could be decreased.

P-Mo

These two elements are present in the soil as anions; thus, they may compete for the exchange sites on the clay to cause a deficiency. However, Basak et al. (1982) found a positive P x Mo interaction for the content of P, Mo, and Zn

in rice. Doberenier (1978) found that when beans were supplied with Mo and P they were able to obtain all the N necessary to produce 3,000 kg seed ha^{-1} which is five times the average Brazilian yield.

Soil Moisture

Bean plants have a short life cycle and are very sensitive to adverse climatic conditions. Water stress during flowering and pod formation reduces bean yield (Doorenbos and Pruitt, 1977; Halterlein, 1983). This susceptibility is due mainly to the effect on the shallow bean root system, thus beans are particularly responsive to frequent irrigation. Inforzato and Miyasaka (1963) determined that 75% of the roots were in the first 10 cm of soil and 85% were in the first 20 cm. It has been reported that when bean plants are suffering from water stress, the root:shoot ratio increases before flowering but the reverse was true after 80 d due to the cessation of growth and retranslocation of dry matter causing root decay (D'Souza and Coulson, 1988). Menezes and Pinto (1967) found that field capacity is the optimum soil-moisture content for bean crops.

The impact of soil moisture and aeration on nodule formation under field conditions has not been studied in detail. Sprent (1976) stated that water stress as well as water logging reduce nodule number and size, and depressed BNF in beans. Freire (1984) pointed out that moisture is a

critical factor limiting nodulation in beans. Contrary to beans, soybeans can nodulate well in dry soil conditions in which beans do not form nodules. Sinclair et al. (1988) reported loss of nodules in soybean did not occur readily upon the imposition of drought, and severe drought was required before nodule numbers or dry weight decreased. Freire (1984) suggested that the critical moisture range for beans is narrower than for soybeans; this could explain the erratic results of inoculation of beans in field trials, and why good nodulation is commonly found in sandy black soils rich in organic matter. Abdel-Ghaffar et al. (1983) determined that water stress depresses nodulation, nitrogenase activity, and grain yield of bean plants. Espinosa et al. (1985) found a reduction of up to 95% of N_2 fixation when different strains were grown under drought versus irrigated conditions.

There is considerable variation of resistance to water stress among genera and species of legumes. Some bean cultivars can tolerate inundation well; losses ranging from 17 to 70% following prolonged flooding have been reported by Graham (1984). One factor that influences their tolerance to excess water is the ability of the plants to transport oxygen from shoots to roots (Sprent, 1976). Minchin et al, (1985) suggested that oxygen as well as carbohydrate supply to the nodules has to be considered when calculating the rate of N_2 fixation in a plant.

N Fertilization of Maize

Nitrogen is the most critical limiting nutrient for maize production in the humid tropics, and heavy applications are required for maximum yields (Abruna et al. 1975). On the other hand, the less expensive common N sources are often residually acid; this plus the natural soil acidity of the tropics could bring about potential problems.

In Florida, irrigated maize has yielded more than 12,500 kg grain ha^{-1} when not limited by nutrient supply or pest attacks. Recommended fertilization rates are based upon yield goal, plant populations, and soil characteristics (Rhoads, 1983). The frequency of fertilizer application depends on the mobility of the soil nutrients and the uptake of these nutrients by the plant. For irrigated maize, Rhoads (1983) claims that N, P, Ca, and Mg are important throughout the growing season, while the other elements are more important during tasseling.

Sulfur

Sulfur requirement in plants is closely related to N metabolism, and high application of N fertilizer to increase crop production may be detrimental and often wasteful if the corresponding increase in S is not met. Stryker (1978) reported that S deficiency may be limiting yields in Honduras. Similar observations were made by Walters (1984, personal communication) for Zamorano soils, and in El

Salvador (Arias, 1985). Sulfur deficiency frequently occurs in soils derived from volcanic parent materials. In such soils, which are common in Central America (Fritts, 1970), the organic matter is closely associated with allophane and the mineralization of the allophane-bound organic matter (the rate of release of sulfate) is very low. Plants in these soils are commonly S deficient despite the soil's high organic matter content.

In Central America, maize is grown mostly without irrigation and without the necessary inputs to obtain high yields. Widespread use of low-yielding cultivars of maize has also been pointed out as a limitation that reduces the possibility of increasing crop yields (Arias, 1985). Due to lack of resources, low yielding cultivars are also accompanied by low amounts of inputs, in general, including N.

An inexpensive fertilizer that supplies both N and S and is commonly used in the area is ammonium sulfate. However, the potential damage that this fertilizer may cause when applied together with triple superphosphate (TSP) to the maize seedlings has been reported by Jacome and Blue, (1980). Based on their work with potted plants, they determined that the salt concentration formed in the soil when both fertilizers were applied did not affect germination compared to TSP applied alone, but the salt concentration adversely affected plant height, plant dry

weight, nutrient contents, and plant roots 33 d post planting. Shoot dry weight and root dry weight were reduced 50 and 40%, respectively. They asserted that root dry weight could be affected more severely under field conditions where plants are subjected to uneven rainfall distribution. Deep roots are necessary not only to support the plant but for the uptake of water and nutrients.

CHAPTER 3
GENERAL INFORMATION ON THE EXPERIMENTS

Maize-Bean Production Systems

In Latin America, 80% of beans are produced in association with other crops, especially maize and sorghum (Francis, 1978). In Central America there are two maize-bean growing seasons. The first one (primera) begins when the rainy season starts in May or June. Beans are planted in monoculture or interplanted with maize. Beans produced in this season are largely used as seed for the second (postrera) season which begins in the September-October period. Most of the beans grown in this season are planted in relay with maize. In this system, common throughout Latin America, beans are planted into maturing stands of maize toward the end of the growing season. Maize plants provide support for the climbing beans.

According to CONSUPLANE (1981), 50% of bean production in Honduras comes from the 53% of farms smaller than 10 ha. By contrast, only 4% of bean-producing farms are larger than 50 ha, and they produce 14% of the total production.

Soils

Most Central American soils are derived from volcanic ash and are characterized by great variability. Muller et al. (1968) characterized 110 soils from Guatemala, El Salvador, Honduras, and Nicaragua. These are compared in Table 3-1 with the characteristics of Honduran soils.

In another study, Diaz-Romeu et al. (1970) found that 69% of 167 soil samples from Central America contained between 0.11 and 0.40% N. The majority (75%) of the soils studied were deficient in N and plants in those soils responded to N application. Fifty-seven percent of those soils had a C content between 1 and 2.5%. They reported C/N ratios that averaged 10:1. They found significant correlations among rainfall, ecological formations, pH values, and organic matter.

Honduran soils have an ustic moisture regime, thus they have 90 accumulative dry days per year. Differences in average soil temperatures (50 cm-depth) between rainy season and dry season are less than 5 ^{0}C and annual average soil temperature is equal to or exceeds 22 ^{0}C (van Wambeke, 1984).

Table 3-1. Selected characteristics of soils from Central America, compared to soil characteristics from Honduras (Muller et al. 1968).

Soil characteristic	Central America	Honduras
Clay	3-72 %	9-59%
pH (H_2O)	5.5-7.5	5.5-7.5
C	0.6-4.4 %	1.4-3.1 %
N	0.04-0.49 %	0.1-0.24 %
CEC	14-63 cmol kg^{-1}	14-63 cmol kg^{-1}
Base Sat.	41-100 %	80%

Description of the Experimental Area

Experiments were conducted at the Escuela Agricola Panamericana (EAP), situated in the Yeguare (Zamorano) Valley, 37 km east-southeast of Tegucigalpa, Honduras. Elevation is 793 m above sea level. Annual average temperature is 24.4 ^{0}C with the hottest average temperature in May (26 ^{0}C) and the coolest in January (22.5 ^{0}C). The average annual rainfall for the last 15 yr is 1110 mm (Anon., 1989).

The dry season runs from November through April. The rainy season extends from May through October (Appendix B). This rainy period represents 87% of the annual total rainfall. The second maize-bean planting season is initiated in September, the wettest month of the year.

Soils used for these experiments were selected from four areas of the EAP. The following criteria were used to select these soils:

a) low available P,

b) low pH, and

c) different management histories.

Soils from three of the selected sites, Colindres, San Nicolas, and Agronomia, used for these experiments are classified as Typic Ustifluvents. These soils are well drained and have moderate permeability. They are classified

in use capacity class II, meaning they are suitable for production of agronomic crops. Slopes are not greater than 5 %. A fourth soil collected from Horticultura has been classified as a Vertic Haplustalf; slopes range from 0 to 2 %, they have deficient drainage and moderate permeability. Use capacity is class III with problems of excess water (Anon., 1989). Selected properties of the soils used in these experiments are presented in Tables 3-2 and 3-3.

These soils have had very different management histories. Agronomia soils have been planted for several decades with annual crops. Colindres has been planted since 1981 with annual crops, mostly maize and occasionally with sorghum but never with beans. Before 1981, Colindres was planted to pasture. San Nicolas has been planted with cotton, maize, and occasionally with beans for over 50 yr. On the other hand, soils in Horticultura have been intensively used for vegetable crops including green beans (*P. vulgaris* L.) for over 40 yr.

Table 3-2. Selected chemical characteristics of the soils used for the bean experiments in 1982 to 1985 in Zamorano, Honduras.

Soil characteristic	Location				
	Agron. 82	Hort. 82/3	Col. 83	Col. 84	Hort. 84
Texture	SL	SL	SCL	SCL	SL
pH (1:2.5)	5.5	5.3	5.1	5.6	5.1
O.M. (g kg^{-1})	23	25	35	36	25
P †	17	15	11	6	17
K	468	256	220	285	300
Ca	1180	1180	1300	1569	1030
Mg	124	124	108	135	156
Cu	2	1	2	2	2
Fe	10	12	8	14	8
Mn	44	19	48	39	25
Zn	4	2	4	13	3

† Nutrients are expressed in mg kg^{-1} soil. Extracted with 0.05 M HCL and 0.0125 M H_2SO_4 (Mehlich I) [Mehlich, 1953].

Table 3-3. Selected chemical characteristics of the soils used in the maize experiment in Zamorano, Honduras, 1984.

Soil characteristic	Location		
	Colindres	San Nicolas	Agronomia
Texture	SCL	CL	SCL
pH (1:2.5)	5.3	5.5	5.5
O.M. (g kg^{-1})	19	33	23
P †	26	10	20
K	276	324	380
Ca	1332	1752	1628
Mg	136	232	132
Cu	1	1	1
Fe	20	11	18
Mn	44	33	40
Zn	4	2	3

† Nutrients were analyzed using Mehlich I extracting solution (1953) and are expressed as mg kg^{-1}.

Soil and Plant Analysis

Chemical content of a composite soil sample from each location prior to initiation of the study and soil samples of the field plots before planting and after harvesting was determined by the IFAS Extension Soil Testing Laboratory, University of Florida. Soil nutrients were extracted using Mehlich I solution (0.05 $\underline{M}$ HCl + 0.0125 $\underline{M}$ H_2SO_4). Soil filtrates were analyzed by atomic absorption spectrophotometry, except P which was analyzed using the Technicon Autoanalyzer and K which was determined by flame emission spectrophotometry. Organic matter was determined by Walkley and Black (Rhue and Kidder, 1983). Lime requirements (Adams and Evans, 1962) were determined at the soil laboratory at the Escuela Agricola Panamericana.

Foliar samples were analyzed by the IFAS Extension Soil Testing Laboratory of the University of Florida. Samples were dried at 70 ^{0}C until constant dry weights were obtained and ground to pass a 2-mm mesh screen in a stainless steel Wiley Mill. Samples of 0.2 g were digested to determine total Kjeldahl N (TKN) using the microKjeldahl procedure (Nelson and Sommers, 1973). Plant tissue analysis for determination of P, K, Ca, Mg, Zn, Mn, Cu, and Fe was initiated by ashing 1 g of plant tissue in a preheated furnace (450 ^{0}C) for 3 h. The ashed material was digested

with 20 mL 5 M HCl. The solution was evaporated to dryness and the residue dissolved in 1 mL of 5 M HCl. Deionized water was added to make up to 50 mL (volume). All elements except P were determined by atomic absorption spectrophotometry. Phosphorus was analyzed by the Technicon Autoanalyzer.

CHAPTER 4
BEAN GROWTH AS AFFECTED BY THREE *Rhizobium* INOCULANTS, RATES OF LIME AND Mo, AND COMBINED N

Introduction

Beans are grown extensively without fertilization in Central America. Although high populations of native soil rhizobia are found in Central American soils (CIAT, annual report, 1980), they do not successfully associate with bean plants; this results in low N_2 fixation. Furthermore, in many cases tropical soils are subject to environmental factors such as water stress and high temperatures that do not allow an effective symbiosis. Under such conditions, a large amount of inoculant of competitive and highly effective strains of rhizobia is needed to counteract the aggressive native rhizobia (FAO, 1984b) and to compensate for the rigors of the tropical environment. Applications of larger than normal amounts have been reported to improve BNF (Smith and del Rio Escurra, 1982). An amount five times greater than that recommended for small seeds such as beans has given better results in Florida than in seeds inoculated with lower amounts. This is to compensate for the extreme

climatic conditions such as high temperature prevalent in Florida. The extra inoculant could be an inexpensive way to ensure against inoculant failure (Hubbell et al., 1989).

Response of beans to inoculation, especially in field trials, has been extremely variable due to many poorly understood limiting factors (Graham, 1981; Barkdoll et al., 1983). Some authors suggest that host plant characteristics are among the principal limiting factors of BNF (Graham and Rosas, 1977; Graham, 1984). Many authors contend that the bacteria and environmental factors also play an important role (Freire, 1984; Graham, 1981). This is especially important in tropical soils, where response to inoculation is often site specific (Wynne et al., 1987), and where there is a great variability of soils and microenvironments directly affecting the performance of the host-bacteria association and BNF.

Sartain et al. (1983) found large differences in bean yield caused by cultivar differences, site environment, and planting season. CIAT Rhizobium strains produced higher bean yields than Nitragin strains in one location. However, in another location, the opposite results were obtained. Barkdoll et al. (1983) reported that inoculation with Rhizobium produced yields that were comparable to 60 and 200 kg N ha^{-1} in two Zamorano sites. Furthermore, they found that Honduran strains and Nitragin strains produced higher yields than CIAT strains.

Two of the nutrients which are very important for BNF in tropical soils are Ca and Mo. Beans failed to obtain the needed Mo for BNF in acidic soils of Brazil (Franco and Day, 1980). Availability of Mo is pH dependent. On the other hand, responses to lime are attributed to a greater availability of Mo at higher pH (Anderson, 1956). Without lime, application of Mo had little effect on plant N or plant growth in acidic soils of Brazil (Franco and Day, 1980). Either applications of high levels of lime (to increase pH to 6.0) or lower levels of lime complemented with Mo seem to be necessary for effective BNF. These authors suggested that in slightly acidic or neutral soils, application of only Mo will be sufficient to solve the problem. They also found that liming to a pH above 6.0 increased plant growth and BNF.

Results are reported here of an initial experiment conducted to determine the value of exotic versus indigenous Rhizobium strains for use as bean inoculants. Strains proven to be good N_2-fixers were provided by Nitragin Co. (Wisconsin, USA) and CIAT (Colombia) for comparison with indigenous Honduran strains. Strains were tested with four treatments of lime and two of Mo using two commercial cultivars. A second experiment was conducted with two soils, using granular form of the same Rhizobium strains than the first experiment, and tested with two commercial cultivars.

The objective of this study was to validate the behavior of the strains in the two soils under study.

Materials and Methods

1982 Experiment

Strains of *Rhizobium leguminosarum* biovar. *phaseoli* tested included a mixture of CIAT 1057 and CIAT 75; implant "D" from Nitragin Company, and a mixture of Honduran 258, 259, and 260, indigenous *Rhizobium* isolated in 1981 (Barkdoll et al., 1983). All *Rhizobium* strains were prepared as seed coatings and granular forms were provided by Dr. S. Smith, from Nitragin Company, Milwaukee, WI.

Two cultivars were used. Acacias 4 is a bushy, semi-determinate cultivar specific for monoculture. It flowers at 38 to 40 d and matures around 68 to 72 d. Seeds are opaque red. Porrillo 70, introduced to Honduras, is a black-seeded semi-determinate bush with short guides; it matures at around 75 d. Porrillo 70 was obtained from F. Bliss (University of Wisconsin). It had been previously shown to be active in N_2 fixation when inoculated with appropriate strains (Sartain et al., 1983, Barkdoll et al., 1983). Both cultivars were grown in an acidic soil collected from the Agronomia site. The experiment was conducted in June 1982. Soil properties of Agronomia (Typic Ustifluvent) are given in Table 3-2.

Soil pH as determined in a 1:2.5 soil:water by volume ratio was 5.5. Lime requirement was determined by the method of Adams and Evans (1962). Four treatments [0, 1.2, 2.4, and 3.6 cmol ½ $Ca(OH)_2$ kg^{-1}] of reagent quality were applied 10 d prior to planting. Water was added immediately after applying the lime. The soil was mixed once daily to insure a homogeneous contact of the lime with the soil.

A 3-cm deep layer of washed gravel covered with filter paper was placed in the bottom of plastic pots of 3-kg capacity, then 2-kg of soil were added to each pot. A solution containing all nutrients except N, Ca, and Mo was applied 1 d before planting. One hundred and fifty milliliters of water were applied daily to each pot. Water was applied twice a day toward the end of the growing period.

Eight grams of each peat inoculant per 100 g of seed were used with 2.5 mL of gum arabic to coat the seeds. The seeds were put in a plastic bag with the inoculant and mixed well until it appeared that they were homogeneously covered by the inoculant (NifTAL, 1980). Five seeds were placed in each pot in June 1982. Eight days after planting, stands were thinned to two seedlings per pot. Pots were arranged in a completely randomized design. A factorial combination of three inoculants, two cultivars, and four lime treatments using $Ca(OH)_2$ was employed with four replications.

Molybdenum was applied at the rate of 0 and 2.4 mg per pot as sodium molybdate reagent quality along with 2.4 cmol ½ $Ca(OH)_2$ kg^{-1} to determine the effect of the Mo on bean growth. Pots were rotated once a week. Plants were harvested at R6 stage (50% flowering). Nodules were separated from roots, and nodule dry weight recorded. Plant tops were dried at 70 ^{0}C until constant dry weight was obtained.

Statistical analysis was conducted using SAS programs (SAS, 1987). This experiment was first analyzed as a completely randomized design with 24 treatments without including the Mo treatments. The presence of these last treatments required a second analysis to compare treatments with 2.4 cmol ½ $Ca(OH)_2$ kg^{-1} with and without Mo. Regression analysis to determine lime response was conducted with the first set of data mentioned above. Orthogonal contrasts were used to compare bean plant response to indigenous versus exotic strains.

1985 Experiment

Strains used in this experiment were the same as in the first experiment. The granular form of inoculants was used. The amount of inoculant used (0.12 g per pot) was ten times that recommended by Nitragin Co.

Acacias 4 and Porrillo Sintetico, two commercial cultivars, were planted in clay pots using two soils, Colindres and Horticultura (2.6 kg pot^{-1}). Colindres soil has been classified as a Typic Ustifluvent. Horticultura

soil has been classified as a Vertic Haplustalf (Anon., 1989). Soil characteristics are presented in Table 3-2. A treatment with 50 mg N kg^{-1} as NH_4NO_3 (35% N) was included in order to compare combined N versus symbiotically fixed N. A nutrient solution containing all nutrients was applied before planting. Lime and Mo treatments were not included in this experiment. The experiment was planted in February 1985. Procedures for planting, maintaining, and harvesting were similar to the first experiment. Bean and nodule weights were recorded, as well as total N. Treatments, including two soils, two cultivars, and four N sources (three strain mixtures and one chemical N) were replicated four times.

Statistical analyses were conducted using SAS procedures (SAS, 1987). The experiment was analyzed as a completely randomized design including the N treatment. Orthogonal contrasts were used to determine differences between exotic and indigenous strains. The Waller-Duncan Test was used to compare means of N sources.

Results and Discussion

1982 Experiment

Because the experiment was exposed to the outside environment, plant growth was affected by compaction of the soil due to rainfall. As a result, low plant dry weights were obtained. Plant dry weight is presented in Table 4-2.

Collected nodules were damaged by slugs during drying, making it impossible to analyze nodule dry weight.

The analysis of variance showed that the main effects of cultivars and lime were the primary factors influencing growth (Table 4-1). Plant dry weight as influenced by cultivar is presented in Table 4-2.

The CIAT strain mixture is known to be highly efficient at fixing N_2 in the tropical environment; hence, it is able to induce an efficient translocation of fixed N to the grain as has been reported for some Rhizobium strains by Hungria et al. (1985). Implant "D" from Nitragin has also proved to be a good N_2-fixer. Although all inoculants produced nodulation in this experiment, CIAT and Nitragin (exotic strains) did not produce plant dry weights different from the indigenous (Honduran) strains. This implies that the Honduran strain mixture can compete with the introduced ones as was found by Barkdoll et al. (1983). The Honduran strains were collected by J.B. Sartain in 1981 from the same soil used in this experiment. This could mean that these indigenous strains are "acclimatized" to this soil environment and are competitive with the introduced ones.

Bean cultivars have been shown to be differentially influenced by inoculation and to interact with Rhizobium strains (Graham and Halliday, 1977; Ramos and Boddey, 1987). However, in this experiment, there was no interaction between them, and only cultivar differences were observed

(Table 4-2). Acacias 4 cultivar produced higher plant dry weight than Porrillo 70 cultivar. This was expected since Acacias 4 is an improved cultivar that was developed from improved Honduran strains of genetic material.

Table 4-1. Analysis of variance of bean plant dry weight from the Rhizobium strain experiment in Honduras in 1982.

Source of variation	df	M S	F
Cultivar	1	0.87	**
Inoculant	2	0.12	NS
Cultivar*Inoculant	2	0.16	NS
Lime	3	0.58	**
Cultivar*Lime	3	0.11	NS
Inoculant*Lime	6	0.04	NS
Cultivar*Inoculant*Lime	6	0.15	NS
Error	72	0.09	
Total	95		
C.V. (%)		19	

** Significant at the 0.01 probability level. NS=Not significant.

Table 4-2. Effect of cultivars on bean plant dry weight from the Rhizobium strain selection experiment in Honduras in 1982.

Cultivar	Mean
	Plant dry wt (g pot^{-1})
Acacias 4	1.65
Porrillo 70	1.48
$LSD_{0.05}$	0.08
C.V. (%)	19

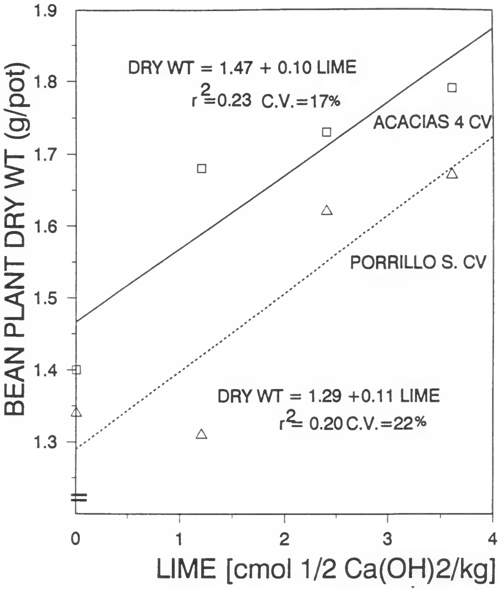

Fig. 4-1. Bean plant dry weight response to lime treatments for the inoculant experiment in Honduras in 1982.

Despite the fact that the soil in the unlimed pots was not very acidic (pH 5.5) there was a significant increase in plant dry weight with liming (Fig. 4-1). Similar responses between pH 5 and 6 have been reported earlier for nodulation and plant dry weight of beans (Munns et al., 1977; Franco and Day, 1980; Da Paz et al., 1982). Addition of 1.5 times the amount required to increase the pH to 6.5 according to the Adams and Evans buffer method (1962) did not increase the pH to more than 6.4.

This positive response to lime was expected since Rhizobium, as all bacteria, is favored by near neutral soil reaction. Also, bean plants grow better at higher pH. However, the low (<0.25) coefficient of determination suggested that other factors influenced plant dry weight. Compaction of the soils due to the rainfall could be the reason for the low correlation of the response to the treatments.

Incremental increases of plant dry weight in response to the addition of lime could be the result of more S, P, and Mo availability for the plant and to the reduction of potential Al and Mn toxicities. However, there was no interaction between lime and Mo in this experiment. Plant dry weight was increased from 1.68 to 1.88 g pot^{-1} when 2.4 mg Mo per pot were applied together with lime but the difference was not significant, suggesting that the amount of applied lime was enough to release the soil Mo needed or

that seeds from these two cultivars have enough Mo reserves and this element was not deficient.

1985

Application of a larger amount of *Rhizobium* (0.12 g per pot) combined with better growing conditions in the glasshouse resulted in higher yields than were observed in the first experiment. An analysis of variance of the data is presented in Table 4-3.

Response to strain mixtures and chemical N was influenced by soils and cultivars. In the Colindres soil (Fig. 4-2), Acacias 4 cultivar responded equally to the inoculants and combined N. Orthogonal contrasts between indigenous and exotic strains were not significant (at the 0.05 probability level). This means that any of the three strain mixtures could be used to inoculate Acacias 4 cultivar in this soil. In the case of the Porrillo Sintetico cultivar, combined N induced more plant dry weight than did the inoculants. In this case, application of the CIAT-strain mixture resulted in inferior plant dry weight production. However, when the response to indigenous strains was compared with the average of the exotic strains using orthogonal contrast (0.05 probability level), indigenous and introduced strains behaved similarly.

Table 4-3. Analysis of variance of bean dry weight in response to N source from the inoculant experiment in Honduras in 1985.

Source of variation	df	Plant dry wt	
		MS	F
Soil	1	58.83	**
Cultivar	1	0.88	NS
Soil*Cultivar	1	15.20	**
N source	3	13.84	**
Soil*N source	3	7.75	**
Cultivar*N source	3	15.36	**
Soil*Cultivar*N source	3	7.72	**
Error	48	1.05	
Total	63		
C.V. (%)		13	

** Significant at 0.01 probability level. NS=Not significant.

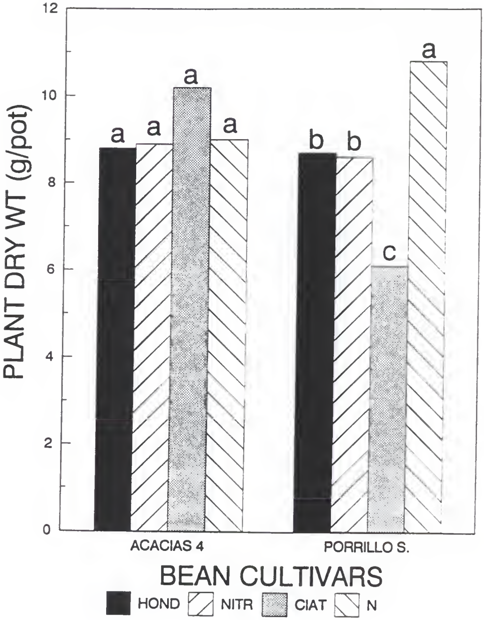

Fig. 4-2. Bean plant dry weight as a response to N source in Colindres soil. Rhizobium inoculation experiment in Honduras in 1985. (Plant dry weight responses to N source within cultivars with the same letter above the bars are not significantly different by Waller-Duncan Test, at the 0.05 probability level).

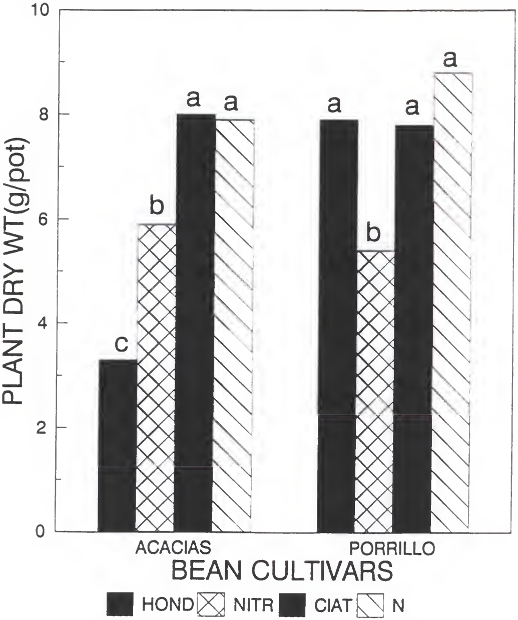

Fig. 4-3. Bean plant dry weight as a response to N source for Horticultura soil. Rhizobium inoculant experiment in Honduras in 1985. (Plant dry weight responses to N source within cultivars with the same letter above the bars are not significantly different by Waller-Duncan Test, at the 0.05 probability level).

In the Horticultura soil, average plant dry weight was lower than in the Colindres soil. The Acacias 4 cultivar inoculated with the CIAT-strain mixture resulted in plant dry weight similar to that obtained with combined N (Fig. 4-3). Dry weight of plants with the Nitragin-strain mixture was lower but not as low as the plants inoculated with the indigenous strains; the latter produced half of the dry weight of the CIAT and combined N. An orthogonal contrast showed that introduced strains were superior to the native ones (0.05 probability level). For Porrillo S. cultivar, plant dry weight responses to CIAT and Honduran-strain mixture were not different from the response to combined N. Plant dry weight with the Nitragin-strain mixture was lower than weight of plants grown with the other three N sources. However, orthogonal contrasts did not show a difference between native and introduced-strain mixtures (0.05 probability level).

Even though the CIAT inoculant produced higher responses in some cases, response to the inoculants were not different, as was demonstrated by Waller-Duncan Test. In three out of four cases, orthogonal contrasts showed no difference between introduced and native strains. In three out of four combinations of cultivars with soils, combined N resulted in higher bean dry weight than inoculants. Only in the case of Acacias 4 cultivar in Colindres soil was the

bean dry weight response to inoculant not different from the combined N treatment.

Total N has been reported to increase when plants are fertilized with combined N (Huntington et al., 1986; Evans, 1982). However, in this experiment, total N was similar for the different N sources, suggesting that inoculation was able to produce similar amounts of total N in bean plants as combined N did. Plant dry weight and total N content were not correlated; this indicated that other factors influence plant growth in addition to N sources. Due to this, N was omitted from this discussion.

Conclusions

Results from the 1982 and 1985 experiments showed some trends in response to strain application. In both experiments, the CIAT inoculant produced higher dry weight in Acacias 4 cultivar. On the other hand, for the two Porrillo cultivars, the inoculant containing the indigenous strains worked better. The association of the Porrillo cultivars with strains from CIAT was not effective as shown in data from the 1982 experiment and in 1985 Colindres soil; both soils have been classified as Typic Ustifluvent.

This variable influence of the inoculants on bean parameters is common in Central American soils as shown by the results of Sartain et al. (1983), and Barkdoll et al. (1983). Variation due to site, cultivar, and strain has been

reported by Halliday (1984) and Wynne et al. (1987). These interactions have been pointed out as a possible complication for the selection of genotypes to improve fixation rates (Graham, 1981).

Use of more than one strain mixture has been recommended by several authors. Graham (1981) and Halliday (1984) mentioned that the use of a single-inoculant strain, as practiced by some authors, could be inappropriate for beans in which strain competition for nodulation sites has been mentioned as a major field problem. In these studies no strain mixture was superior to the other strains. Based on the results reported herein it was decided to use a mixture of the three inoculants (exotics + native) for subsequent experiments with Honduran soils to determine the roles of lime, P, and Mo on BNF and bean growth.

In the 1982 experiment, lime response was linear; the highest plant dry weight was obtained at the highest lime level that corresponded to pH 6.4. Although the application of Mo increased the plant dry weight, this increase was not significant; probably amounts of available Mo increased with lime application and/or the seeds contained enough for bean growth.

Growth of inoculated bean plants was never better than plants which received combined N. This would indicate that even the best candidate strains of Rhizobium were not able

to provide adequate quantities of N for bean growth consistently.

CHAPTER 5
EFFECT OF LIME AND N SOURCE ON BIOLOGICAL NITROGEN FIXATION AND BEAN GROWTH

Introduction

Beans are grown in every Central American country, even though cultivation of this legume is not easy in the predominantly acidic, tropical soils. Many tropical soils in Latin America have a pH lower than 6.5. In most cases, the adverse effects of acidic soils on legumes and *Rhizobium* cannot be attributed to the hydrogen ion concentration itself, but rather to deficiencies of P, Ca, Mg or Mo, or to the toxic effects of soluble Al and Mn (Freire, 1984).

Rhizobium strains seem to be more resistant to the acidic environment than are the plants. Sand-culture data reported by Franco and Munns (1981) showed that nodulation of *P. vulgaris* L. is particularly sensitive to acidic conditions. Liming of the soil can alleviate problems related to soil acidity, but addition of lime and fertilizers to some tropical soils, particularly in virgin soils, may produce a microbial flora antagonism to *Rhizobium* (Scotti et al., 1982); liming may also cause some nutrient deficiencies. Glasshouse experiments were conducted to

determine optimum pH for biological N_2 fixation and bean growth in Honduran soils.

Materials and Methods

1983 Experiment

A glasshouse pot experiment was conducted in September 1983, to test soils from two sites, Colindres (plot 1) and Horticultura (plot 21). Chemical characteristics of these soils are presented in Table 3-2.

The amount of lime necessary to increase soil pH to 6.5 was determined by the method of Adams and Evans (1962). This method had been previously determined to be the most suitable for Zamorano soils (J. Escamilla, unpublished). Lime was applied 10 d prior to planting at the following rates corresponding to 0, 0.5, 1, 1.5, and 2.0 times the recommended amount of lime. For the Colindres soil, lime levels were 0, 1.07, 2.14, 3.21, and 4.28 cmol ½ $Ca(OH)_2$ kg^{-1}. For the Horticultura soil the levels were 0, 1.10, 2.20, 3.30, and 4.40 cmol ½ $Ca(OH)_2$ kg^{-1}. Reagent quality $Ca(OH)_2$ was the source of lime. The soil was moistened and thoroughly mixed daily.

Two kilograms of the pH-adjusted soil were transferred to 3-kg plastic pots and mixed with a nutrient solution according to recommendations of Diaz-Romeu and Hunter (1978) (Appendix A). Only N was omitted from this solution.

Seeds were coated with peat inoculant containing equal parts of the indigenous (Honduran) and exotic (Nitragin and

CIAT) strain mixtures. Five seeds of one cultivar - either Acacias 4, Porrillo 70 or Line 1258 (Bliss 2) - were planted in each plastic pot. After 1 wk, plants were thinned to two per pot. Water was initially applied at the rate of 150 mL pot^{-1}, increased to 200 mL pot^{-1} the second week, and 300 mL pot^{-1} subsequently. Plants were harvested at R6 stage (50% flowering), dried at 70 ^{0}C until constant weights were obtained, then ground and analyzed. Dry weight of plants, roots, and nodules were recorded.

A completely randomized factorial arrangement of the two soils, three cultivars, and five lime levels was used. Each treatment was replicated three times. Statistical analyses were calculated using the SAS program (SAS, 1987).

1984 Experiment

A second glasshouse pot experiment was established in May 1984 at the Escuela Agricola Panamericana. Three centimeters of washed gravel were placed at the bottom of clay pots. A filter paper was laid on top of the gravel. Colindres and Horticultura soils were added at the rate of 2.6 kg pot^{-1}. Nutrient solution was applied in the same way as in the previous experiment. A treatment of 50 mg N kg^{-1} using reagent quality NH_4NO_3 (35.0 % N) was included in the experiment in order to compare the effect of the inoculant with that of combined N. Lime levels for the Colindres soil were 0, 0.94, 1.87, 2.81, and 3.74 cmol ½ $Ca(OH)_2$ kg^{-1}. For

the Horticultura soil lime levels were 0, 0.96, 1.92, 2.88, and 3.84 cmol ½ $Ca(OH)_2$ kg^{-1}.

Acacias 4 and Porrillo Sintetico cultivar were used for this study. Granular inoculant was applied alongside the five seeds at a rate of 0.12 g per pot. After 1 wk, plants were thinned to two per pot. Data recorded were plant and nodule dry weights and total N content of bean plants at R6 stage.

The experiment was set up as a completely randomized design with four replicates. Statistical analyses were calculated using the SAS program (SAS, 1987). An initial analysis was calculated with only the treatments containing inoculant. A second analysis was carried out considering the treatments that included the recommended lime levels for both soils with strain inoculation and with N fertilization.

Results and Discussion

1983

Soils used in this study are not extremely acid and had been reported to be low in exchangeable Al (Anon., 1989). Increasing levels of lime produced the following soil pH: for the Colindres soil pH values were 5.1, 5.9, 6.0, 6.4, and 6.9 for lime rates 0, 0.5, 1, 1.5, and 2.0 times the lime rates required to raise pH to 6.5, respectively. Similarly, for the Horticultura soil, pH was increased to 5.4, 5.5, 5.8, 6.1, and 6.9. The effect of lime on bean

growth was measured by the plant, nodule, and root dry weight responses. Analysis of variance and results are presented in Tables 5-1 and 5-2.

Bean plant dry weight. The Waller-Duncan Test showed that plant dry weight was influenced by cultivar (Table 5-2). Porrillo 70 cultivar produced superior plant dry weight in relation to Acacias 4 cultivar and line 1258 (Bliss 2). Neither lime applications nor soils affected bean plant dry weight.

Nodule dry weight. There were no differences in nodule dry weight due to the lime treatments nor due to soils. Nodule weight was only influenced by cultivar. Orthogonal contrasts ($p=10\%$) were used to compare means among cultivars. This analysis showed that higher nodule dry weight was produced by the line 1258 than by Porrillo (Table 5-2). The nodule dry weight of Acacias 4 cultivar was not different from the ones produced by line 1258.

Root dry weight. Soil type and lime influenced root dry weight. Mean root dry weight of the three cultivars grown in the Colindres soil was higher than in the Horticultura soil. Lime effect was linear for Colindres soil (Fig.5-1). Increasing amounts of lime increased pH from 5.1 to 6.4 and root dry weight; however, the correlation between lime and root dry weight was low. In the Horticultura soil, pH increased from 5.4 to 6.9, and there was not a response to lime.

Table 5-1. Analysis of variance of bean plant dry weight, nodule dry weight, and root dry weight as a response to treatments from the lime experiment in Honduras in 1983.

Source of variation	df	Dry wt		Nodule wt		Root wt	
		MS	F	MS	F	MS	F
Soil	1	0.59	NS	10978	NS	0.28	**
Cultivar	2	0.82	**	14884	*	0.06	NS
Soil*Cultivar	2	0.03	NS	13286	NS	0.01	NS
Lime	4	0.14	NS	7568	NS	0.14	**
Soil*Lime	4	0.16	NS	9975	NS	0.04	NS
Cultivar*Lime	8	0.22	NS	11656	NS	0.04	NS
Soil*Cultivar*Lime	8	0.13	NS	8930	NS	0.06	NS
Error	60	0.16		4416		0.04	
Total	89						
C.V. (%)		23		36		29	

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

Table 5-2. Influence of cultivar on bean plant and nodule dry weight from the lime experiment in Honduras in 1983.

Cultivar	Mean	
	Plant dry wt (g pot^{-1})	Nod. dry wt (mg pot^{-1})
Acacias 4	1.57	181
Porrillo 70	1.89	164
Line 1258	1.68	191
<u>CONTRASTS</u>		
Acacias vs. Porrillo	**	*
Acacias vs. Line 1258	NS	NS
C.V.(%)	23	36

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

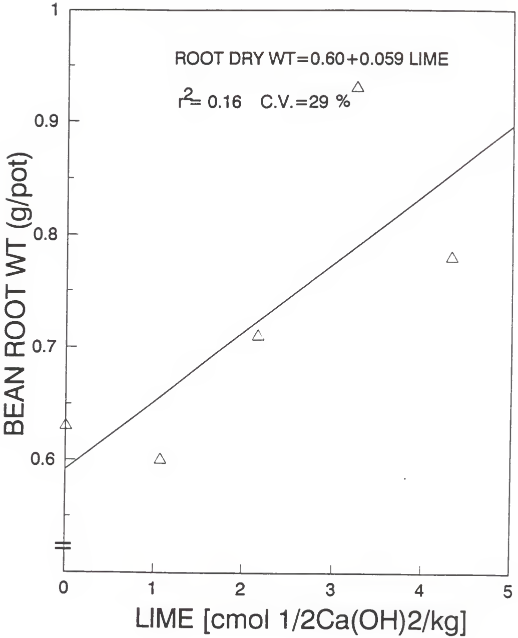

Fig. 5-1. Influence of lime on bean root dry weight. Average of the three cultivars from the lime experiment in Honduras in 1983.

These results may be caused by the differences in soil histories. Plants growing in Horticultura soil are prone to have more nutritional imbalances and water retention problems than in Colindres soil.

Correlation between plant dry weight and nodule dry weight was not significant (at the 0.05 probability level). Correlation between root dry weight and pH was found to be 0.34 with P=0.02 for Colindres soil.

1984

General better conditions in this experiment resulted in higher plant dry weight compared to the 1983 lime experiment. The Colindres soil pH measured was 5.6, 5.6, 5.8, 6.3, and 6.4 when limed with 0, 0.5, 1, 1.5, and 2 times the recommended amount to increase pH to 6.5. Similarly, for Horticultura, the following pH levels were obtained: 5.3, 5.9, 6.2, 6.5, and 6.4. An analysis of variance of the data is presented in Table 5-3. Lime effects on bean plant and nodule dry weight, as well as total N are presented in Figs. 5-2 to 5-4.

Bean plant dry weight. This parameter was influenced by the interaction of lime and cultivar and by soil and lime. The effect of lime on Acacias 4 cultivar plant dry weight was linear (Fig. 5-2). Increases in dry weight were obtained with increasing levels of lime. The low coefficient of determination indicates that some factors other than the ones studied affected plant dry weight. Lime levels may have

Table 5-3. Analysis of variance of plant dry weight, nodule dry weight, and total N from the lime experiment in Honduras, 1984.

Source of variation	df	Dry wt		Nod. wt		Total N	
		MS	F	MS	F	MS	F
Soil	1	15.17	**	17731	**	174.05	**
Cultivar	1	0.29	NS	3162	**	76.05	*
Soil*Cultivar	1	0.66	NS	1386	*	4.05	NS
Lime	4	0.73	**	1495	**	40.33	NS
Soil*Lime	4	1.97	**	508	NS	31.77	NS
Cult*Lime	4	0.70	**	941	*	15.14	NS
Soil*Cult*Lime	4	0.52	NS	763	*	31.46	NS
Error	60	0.22		293		14.88	
Total	79						
C.V. (%)		13		6		12	

*, ** Significant at 0.05 and 0.01 probability levels, respectively. NS=Not significant.

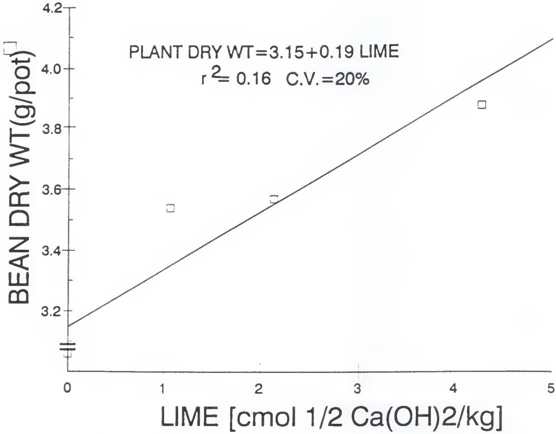

Fig. 5-2. Effect of cultivar by lime interaction on Acacias 4 bean dry weight from the lime experiment in Honduras in 1984.

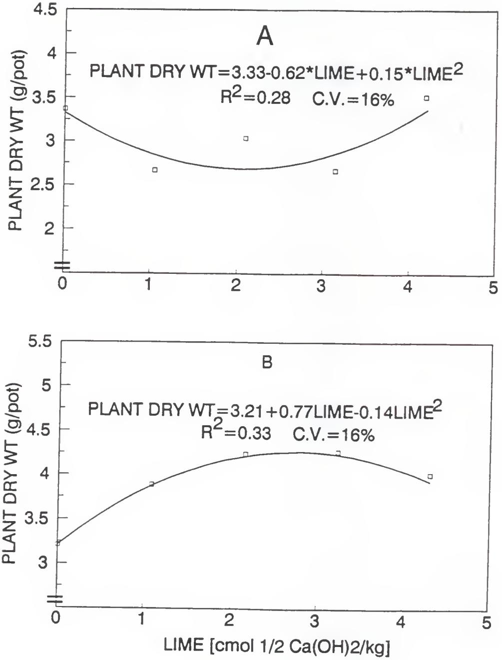

Fig. 5-3. Effect of soil by lime on bean dry weight. Average of the two cultivars from the lime experiment in Honduras in 1984. Fig. 5-3A shows response of bean plants in Colindres soil. Fig. 5-3B shows response of bean plants in Horticultura soil.

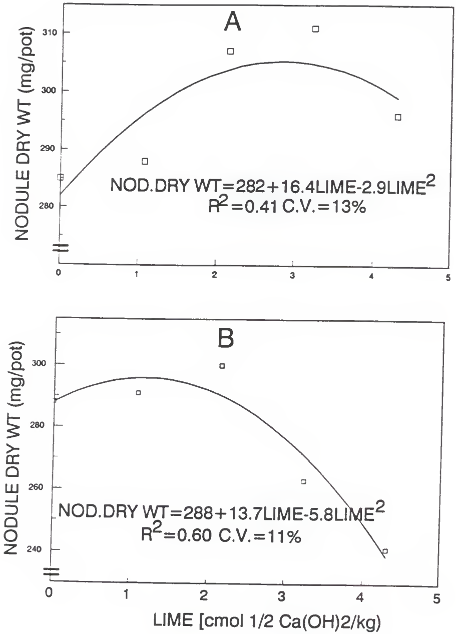

Fig. 5-4. Nodule dry weight as influenced by the interaction of soil, cultivar, and lime from the lime experiment in Honduras in 1984. Fig. 5-4A shows data from Acacias 4 cultivar in Horticultura soil. Fig. 5-4B shows nodulation data from Porrillo Sintetico cultivar in Horticultura soil.

caused an imbalance of micronutrients. In this particular case, lime application caused a decrease of N, Cu, Mn, Zn, and an increase of Ca, Mg, and Mo in bean plants for both cultivars. Correlations of these nutrients in soil and foliar samples with plant dry weight are presented in Appendix G and I. The effect of lime on Porrillo S. cultivar plant dry weight did not fit a response curve. Although plants were green and healthy, well nodulated, and contained sufficient levels of Ca, Fe, Mn, Zn, and P; they were deficient in N, Cu, and Mg according to the bean nutrient contents reported by Howeler (1983). The values of these three elements were under the required amounts for optimum bean growth (Appendix E).

The effect of lime on bean plant dry weight was influenced by soil type. For the Colindres soil with an original pH of 5.6 the response was quadratic (Fig. 5-3A). Without the application of lime, plant dry weight was higher and decreased with the application of the three lower levels of lime, but plant dry weight increased with the application of 1.5 times more lime than that recommended by Adams and Evans (1962). This initial decrease could have been caused by changes in abiotic factors such as nutrients as well as biotic such as microorganisms that were overcome at higher lime levels. Mehlich-I extractable Ca levels were increased by lime applications, but Zn and P were decreased.

For the Horticultura soil, bean plant dry weight increased in a quadratic manner in response to lime application (Fig. 5-3B). Maximum plant dry weight calculated from the curve corresponds to 4.3 g pot^{-1} as a result of the application of 2.75 cmol ½ $Ca(OH)_2$ kg^{-1}. This soil had an original pH lower than the Colindres soil. Soil pH increased from 5.3 to 6.4. Maximum plant dry weight was obtained with pH in the range of 6.2 to 6.5. This is the optimum pH range for beans reported in the literature (Malavolta, 1976; Schwartz and Galvez, 1980) and it is also favorable for Rhizobium. The positive response of bean plant dry weight to liming could be due to several factors such as improved soil structure that allows better root growth, increased water retention, decreased problems with fungal diseases and greater nutrient availability.

Nodule dry weight. Nodule dry weight was influenced by the interaction of soil, cultivar, and lime (Table 5-3). The effect of the interaction of soil with both cultivar and lime was not significant in the Colindres soil. Nodule dry weight was increased quadratically by increasing amounts of lime for both cultivar in Horticultura soil. Acacias 4 cultivar was more responsive to lime applications. Maximum nodule dry weight for Acacias cultivar was obtained when lime was applied at a rate of 2 cmol ½ $Ca(OH)_2$ kg^{-1} corresponding to a pH of 5.9 (Fig 5-4A). For Porrillo S.

cultivar, maximum nodule dry weight was obtained with one cmol ½ $Ca(OH)_2$ lime kg^{-1} (Fig. 5-4B). Application of higher levels of lime caused a decrease in nodule dry weight. Lime pelleting of seeds of tropical species in acidic soils in Colombia showed differences in response of two bean cultivars (Morales et al., 1973). When inoculant was applied without lime, nodules per plant were almost nil, but with the application of 0.3 Mg lime ha^{-1} both cultivars showed an increased number of nodules per plant. Munns et al. (1977) reported increased nodule numbers with increasing amounts of lime up to pH 6. However, this did not correlated with growth improvement. Variation of nodulation in beans was unrelated to growth.

Total N. Differences in N content and partitioning in different cultivars have been discussed by several authors (Neves and Hungria, 1987) and have been related not only to BNF but also to the grain yield and protein content in the seeds. Graham and Rosas (1977) found faster accumulation of N in leaves of bush bean cultivar than in climbing cultivar.

In this study, both cultivars are semi-determinate bush, and differences in total N were observed. Plant total N content was affected by soil and cultivar (Table 5-4). Bean plants in the Colindres soil contained more N than in the Horticultura soil; this could be have been related to the availability of N already in the Colindres soil that favored N uptake by the plants. Organic matter in the

Table 5-4. Effect of soil and cultivar on total N content from the lime experiment in Honduras in 1984.

Soil	Cultivar: Acacias 4	Cultivar: Porrillo Sintetico	Mean
	---------Plant total N (mg kg^{-1})--------		
Colindres	33.70	31.30	32.30
Horticultura	30.30	28.80	29.55
Mean	32.00	30.05	

CONTRASTS	P	C.V.(%)
Colindres vs. Horticultura soil	**	12
Acacias 4 vs. Porrillo S. cv	*	12

*, ** Significant at 0.05 and 0.01 probability levels, respectively. NS=Not significant.

Colindres soil is higher than the Horticultura soil. Differences in total N content in the cultivars were observed. Acacias 4 cultivar. contained more N than Porrillo S. cultivar. Differences of the cultivars in their N partitioning have been mentioned by Hungria and Neves (1987).

Effect of N Source

Treatments with the recommended amount of lime (Adams and Evans 1962) and using Rhizobium and combined N were analyzed separately from the other treatments. Bean plant and nodule dry weight as well as total N response to N sources were evaluated.

Bean dry weight. An analysis of variance of bean dry weight as influenced by N sources is presented in Table 5-5. Bean dry weight was affected by the interaction of cultivar and N source as well as the interaction of soil and cultivar.

Combined N increased plant dry weight by 23% relative to symbiotic N in both cultivars. Application of combined N always resulted in higher bean dry weight than when inoculants were applied (Table 5-6). Combined N generally produced more vigorous growth and a greater uptake of other nutrients. In this experiment a synergistic effect of combined N and Ca, Mg, Mn, and Zn levels in bean plants was recorded.

Table 5-5. Analysis of variance of bean dry weight, nodule dry weight and N content as a response to N source from the lime experiment in Honduras in 1984.

Source of variation	df	Dry wt MS	Dry wt F	Nodule wt MS	Nodule wt F	Total N MS	Total N F
Soil	1	12.40	**	17391	**	26.3	NS
Cultivar	1	1.14	NS	392	NS	19.5	NS
Soil*Cultivar	1	2.22	*	1104	NS	1.5	NS
N source	1	21.62	**	75078	**	0.8	NS
Soil*N source	1	0.01	NS	10	NS	0.8	NS
Cultivar*N source	1	2.10	*	722	NS	5.3	NS
Soil*Cult*N source	1	0.17	NS	420	NS	9.0	NS
Error	24	0.32		478		13.4	
Total	31						
C.V. (%)		13		9		12	

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

Table 5-6. Bean plant dry weight as a response to cultivar and N source from the lime experiment in Honduras in 1984.

N treatment	Cultivar: Acacias 4	Cultivar: Porrillo S.
	----Plant dry weight (g pot^{-1})----	
Inoculated	3.58	3.71
Combined N	4.64	4.84
CONTRAST		
Inoc. vs. comb. N	**	*
C.V. (%)	16	24

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

Table 5-7. Effect of the interaction of soil and cultivar on bean plant dry weight, from the lime experiment in Honduras in 1984.

	Soil	
Cultivar	Colindres	Horticultura
	-----Plant dry weight (g pot^{-1})-----	
Acacias 4	4.29	5.01
Porrillo S.	3.39	5.16
CONTRASTS		
Acacias vs. Porrillo cv	*	NS
C.V.(%)	29	20

* Significant at the 0.05 probability level. NS=Not significant.

In Colindres soil, Acacias 4 cultivar produced higher plant dry weight than Porrillo Sintetico cultivar (Table 5-7). On the other hand, in Horticultura soil, Porrillo Sintetico cultivar yielded more than Acacias 4. However, this difference is lower than the one obtained in Colindres soil. Orthogonal contrasts showed a difference between the two cultivars in the Colindres soil. However, no difference was found between the two cultivars in the Horticultura soil.

Nodule dry weight. Nodule dry weight was influenced by main effects of soil types and N source (Table 5-8). Similar to the response in plant dry weight, higher nodule dry weight was obtained on the Horticultura soil than on the Colindres soil. Nodule dry weight were higher on inoculated plants than the N-fertilized. Application of combined N as low as 2.5 mg kg^{-1} has been reported to reduce nodulation (Graham and Halliday, 1977; Evans, 1982).

Total N. Combined N has been reported to increase total N content in beans (Ruschel and Reuser, 1973). However, in this experiment, bean total N content was not influenced by any of the imposed treatment sources (at the 0.05 probability level). This implies that inoculant was able to supply N to bean plants in amounts comparable to that obtained with the application of combined N.

Table 5-8. Nodule dry weight as a response to N source and soil from the lime experiment in Honduras in 1984.

Soil	N source: Inoculated	N source: Combined N	Mean	LSD 0.05	C.V. (%)
	-----Nodule dry wt (g pot^{-1})----				
Colindres	258	160	209	19	9.4
Horticultura	303	207	255		
Mean	281	184			
$LSD_{0.05}$	19				
C.V. (%)	9				

Conclusions

In legumes, lime may improve BNF by improving nodule numbers, nodule growth or nodule effectiveness. In these two experiments, a mix of positive and negative responses was observed. Variability of results are rather common in the tropics; as Fox and Kang (1977) suggested there is nothing unique about the soil fertility problems encountered in the tropical areas. Munns (1977) talked about tropical soils with acidic pH but not deficient in Ca and not toxic Al and Mn. The effect of these factors on the legume-Rhizobium association is highly variable, especially in beans.

Although some of the parameters measured differed from one experiment to the other, some trends were noted. There was a lack of response to lime application in the 1983 experiment as measured by bean and nodule dry weight. The only parameter that responded was root dry weight, which was favored by the lime application in Colindres soil. It is possible that soil compaction which resulted from rainfall could have contributed to this lack of response. Yields were roughly half of those obtained in 1984.

In 1984, parameters measured showed low correlation ratios with lime levels. From the quadratic response of plant dry weight and nodule dry weight (Fig. 5-3 and

Fig. 5-4), it can be noted that a pH between 6.2 and 6.5 is the optimum for bean growth and BNF in the Horticultura soil. In the Colindres soil the response was mixed, obtaining the maximum dry weight in 1983 when pH was around 6.4. However, in 1984, lime application did not show a clear effect on plant dry weight and nodule dry weight, provided only occasional marginal benefit with maximum values at pH 5.6 and 6.4.

The effect of combined N treatments was greater than inoculated treatments on the parameters studied. These results reconfirm the claim that beans respond to combined N (Vincent, 1974; Graham, 1981) and that BNF does not provide enough N to the plant. This was also observed in a previous experiment not reported here because it was discarded when all inoculated plants presented symptoms of severe N deficiency except the ones with combined N. However, when the inoculated plants were taken out of the pots healthy, red colored nodules were found.

Nitrogen content in plants is not always correlated with yields. In fact, Amarall (1975) cited by Vose (1983) claimed that high N content in leaves indicates less N available for the economically important grain, and is roughly a measure of efficiency.

CHAPTER 6
EFFECT OF PHOSPHORUS AND N SOURCE ON BIOLOGICAL NITROGEN FIXATION AND BEAN GROWTH

Introduction

The P content in tropical soils is highly variable. Fassbender and Bornemiza (1987) summarized data from different authors; total P varies from 18 mg kg^{-1} in Venezuelan Oxisols and Ultisols to 3300 mg kg^{-1} in volcanic soils from Central America. They attributed these huge differences to the parent material, soil development, and edaphic and ecological factors. Even with the significant amount of total P in Central American volcanic soils, the availability of this element for plants is low due to the high fixation capacity of the soils.

A recent study of Zamorano area determined the presence of volcanic remnant materials in some soils especially those close to the mountains. In the valley, however, soils are alluvial and of recent formation. Most of them are classified as Entisols, with low amounts of Al and an unusually high amount of silica (Anon., 1989). Sierra (1959) studied the Ap horizon of three soil types from Zamorano and found pH ranges from 5.35 to 5.83. Phosphorus extracted with

Mehlich I reagent was from 0.3 to 8 mg kg^{-1}. Awan (1964), working with soils of Zamorano, reported significant increases in yields of maize and green manure crops due to liming and P applications. He reported increases of 86% in bean production when lime and P were applied without N. The pH was increased from 5.5 to 6.5 with lime application. Burgos (1967), working with black clay soils of Zamorano with different management histories, found that responses to N fertilizers were not obtained until a sufficient supply of P was supplied. When P was applied, soil microbial activity increased as evidenced by increased CO_2 evolution. He concluded that the unavailability of P to plants was the major factor which limited plant growth in those soils.

Some legume species are more tolerant of P deficiency than others. Zaroug and Munns (1979) found evidence suggesting that the P requirement of symbiotic plants of <u>Lablab</u> <u>purpureus</u> increased if the soil was acid or if an effective strain of <u>Rhizobium</u> was replaced by a non-effective one.

Plant species require different amounts of P to sustain maximum growth. For some plants 0.03 mg P L^{-1} in the nutrient solution is enough. However, many others require higher concentrations (Fox and Kamprath, 1970). Soybean did not respond to more than 25 kg P ha^{-1} (Jones et al. 1977); no further increase in chickpea (<u>Cicer</u> <u>arientinum</u>) yield was

reported when more than 75 kg P ha^{-1} was applied (Parihar and Tripathi, 1989).

External P requirement (requirement for a given crop) differs for different species of plants. It has been suggested that under certain conditions this amount is a plant characteristic (Fox, 1981). Lopez-Hernandez et al. (1987) reported the external P requirement for cowpea (Vigna unguiculata L.) to be relatively constant when growing in five different soils. This amount was around 1.00 μg/mL of solution. However, cowpea yields varied. They concluded that these large differences in yield were caused by limiting factors other than P. Phosphorus external requirement was much higher than the one obtained by Cassman et al. (1981) in high P-fixing soils of Hawaii. They asserted that these differences could probably be caused by plant variety, mycorrhizae associations, and pot wall effects.

In the range of pH from 4.0 to 6.5, most P in the soil is in $H_2PO_4^-$ form (Mengel and Kirkby, 1987). Above that pH, the dominant form of P is HPO_4^{-2}. The rate of phosphate uptake is also pH dependent. Hendrix (1967) found that at pH 4 bean plants absorbed phosphate at a 10-fold higher rate than at pH 8.7.

Diffusion of immobile nutrients such as P across the rhizosphere is limited. This problem could be accentuated in the tropical environment where P is cited as one of the two most important potentially deficient nutrients, especially

since those legumes which depend on N_2 fixation need more P than legumes using combined N.

Few studies to determine the effect of P on the Rhizobium-bean symbiosis have been reported. There is evidence of genetic differences for level of N_2 fixation and plant growth in bean at low levels of plant available P. A strong positive correlation between P application and BNF has been reported by Graham and Rosas (1979). Reported herein are two experiments carried out in 1983 and 1984 to determine effects of P fertilization and N source on bean growth and biological N_2 fixation in soils of Zamorano.

Materials and Methods

1983 Experiment

Soils collected from Colindres and Horticultura were pulverized. A general application of lime (2.14 and 2.20 cmol ½ $Ca(OH)_2$ kg^{-1} for Colindres and Horticultura soils, respectively) was made 10 d before planting. Water was applied immediately to speed the reaction of lime with the soils and every day the soils were mixed. Soil characteristics are presented in Table 3-2. Treatments consisted of 0, 10, 20, and 40 mg P kg^{-1} as monocalcium phosphate. A nutrient solution recommended by Diaz-Romeu and Hunter (1978) (Appendix A) was applied before planting. Phosphorus and N were not added to the nutrient solution.

Plastic pots were filled with 2.0 kg soil. Procedures for the application of strain mixtures, planting, thinning, and harvesting were identical to the 1983 lime experiment (Chapter 5). Acacias 4 and Porrillo 70 cultivar were planted in September 1983. Plants were harvested at flowering stage (R6). Plant and nodule dry weight as well as height of plants were recorded.

The experiment was set up in a completely randomized design with two soils, two cultivars and four levels of P. Each treatment was replicated four times. Statistical analyses were calculated using SAS program (SAS, 1987).

1984 Experiment

Since the black plastic pots used in the 1983 experiment could have produced high temperatures in the soil affecting not only the Rhizobium but also plant growth, in this experiment clay pots were used. Temperatures have been detected to be a major limiting factor for bean growth in tropical soils (Graham and Halliday, 1977). Also, because the lack of response to P in the 1983 experiment may have been caused by high pH due to the lime application, it was decided not to apply lime in this experiment. The experiment was conducted in July 1984. Soils from the same site as the 1983 experiment were used for the 1984 experiment. Chemical characteristics of the soils are presented in Table 3-2. A treatment of 50 mg N kg^{-1} using NH_4NO_3 was included in the

experiment to compare inoculant with combined N. Application of P, nutrient solution, planting, data recording, thinning, and harvesting were identical to the 1984 lime experiment (Chapter 5). Granular inoculant containing equal parts of CIAT (exotic), Nitragin (exotic), and Honduran strain mixture (native), at the rate of 0.12 g per pot on Acacias 4 and Porrillo S. cultivar were used.

A completely randomized design was set up with two soils, two cultivars and four P levels. These treatments were replicated four times. Initially statistical analyses using SAS program (SAS, 1987) were conducted only with those treatments that included inoculants. A second analysis was calculated comparing treatments with inoculant and combined N. In both treatments, 40 mg P kg^{-1} were applied.

Results and Discussion

1983 Experiment

Plants did not grow well, perhaps due to a combination of lack of optimum facilities and the use of black plastic pots. Analysis of variance for this experiment is presented in Table 6-1. Phosphate applications did not have an effect on any of the measured parameters except plant height of Porrillo 70 in the Horticultura soil. This lack of response to applied P could have been caused by the lime application that interfered with the P response. Lime application

Table 6-1. Analysis of variance of plant dry weight and height from the P experiment in Honduras in 1983.

Source of variation	df	Dry weight		Height	
		MS	F	MS	F
Soil	1	1.01	**	8.6	*
Cultivar	1	0.63	*	29.2	**
Soil*Cultivar	1	0.48	NS	0.9	NS
Phosphorus	3	0.06	NS	1.1	NS
Soil*P	3	0.09	NS	2.9	NS
Cultivar*P	3	0.24	NS	2.5	NS
Soil*Cultivar*P	3	0.09	NS	4.3	*
Error	48	0.13		1.5	
Total	63				
C.V. (%)		18		10	

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

increased soil pH of this experiment from 5.1 and 5.4 in Colindres and Horticultura, respectively, to pH 7. Response to the application of a small amount of lime is usually positive on highly weathered soils, because it neutralizes excess exchangeable Al, and Mn, but response at high levels of lime often becomes negative (Sumner, 1979). Three mechanisms are mentioned in the literature to explain what happens when P and lime are applied together resulting in a lime-induced P deficiency. First, various P compounds may be formed by precipitation. A second mechanism involves the adsorption of P onto the surfaces of hydrated Al and Fe oxides and clay minerals. A third mechanism that could be equally important is the one that affects P availability when lime is applied, by influencing microbial activities in soil and thereby the net mineralization or immobilization of soil organic soils (Haynes, 1982). Awan (1964) found P to be in organic form in Zamorano soils.

Bean plant dry weight. Bean plant dry weight were influenced only by the main effects of soil and cultivar. Phosphorus application did not influence bean plant dry weight. Both soils had similar levels of extractable P (11 and 15 mg P kg^{-1}) when the experiment was started. In the Horticultura soil, bean plant dry weight was higher than in the Colindres soil (2.14 vs 1.94 g pot^{-1} $LSD_{0.05}$ *, C.V. 25%). Porrillo 70 cultivar responded more positively than Acacias

4 cultivar (2.16 vs 1.91 g pot^{-1} $LSD_{0.05}$ *, C.V.=25%) to P application.

Bean height. Plant height, have been correlated with yield. Edje et al. (1975) found plant height increased significantly with increase of fertilizer. Bean height as a response to the interaction of both cultivars in Colindres soil and the Acacias 4 cultivar in the Horticultura soil, were not significantly different. However, height of Porrillo 70 cultivar in the Horticultura soil responded quadratically to P application (Fig. 6-1). Maximum plant height was obtained with the application of 25 mg P kg^{-1}. Horticultura soil contained only small amounts of extractable P even though P had been intensively applied.

1984 Experiment

The combination of better environmental conditions and the use of clay pots resulted in higher dry weight production than in 1983 experiment. Data from this experiment are presented in Fig. 6-2. Analysis of variance is presented in Table 6-2.

Bean plant dry weight. Bean plant dry weight was influenced by the interaction of soil by cultivar by P. In Colindres soil, the response to P increased from 1.88 to 6.24 g pot^{-1} when P was applied at 0 and 40 mg P kg^{-1}, respectively). For both cultivars in the Colindres soil and for Porrillo S. in the Horticultura soil a linear regression was obtained with increased amounts of P (Fig.6-2). Original

P content for these soils were low, 6 and 17 mg P kg^{-1} for Colindres and Horticultura, respectively. This latter soil had been fertilized continously with a complete fertilizer; however, in this experiment, bean cultivars in Horticultura soil showed a liner response to P. Vigorous plant growth including root growth has been attributed to P application (Cassman et al., 1980; Graham and Rosas, 1979; Fageria, 1989; Chaib et al. 1984). This could have resulted in a greater absorption of water and nutrients, as well as beneficial to BNF.

The results herein agree with those found by Pereira and Bliss (1987) and Fageria (1989) who reported differences in cultivars responding to P. The latter author found the optimum yield parameters to P levels at 125 to 150 mg P kg^{-1}.

Total N. Bean plant total N was affected by the main effect of cultivar. Total N was higher in Acacias 4 cultivar than in Porrillo S. cultivar. This is probably caused by differences of the hosts in their ability to partition the N. The influence of P applications on bean plant total N was not significant for either soil.

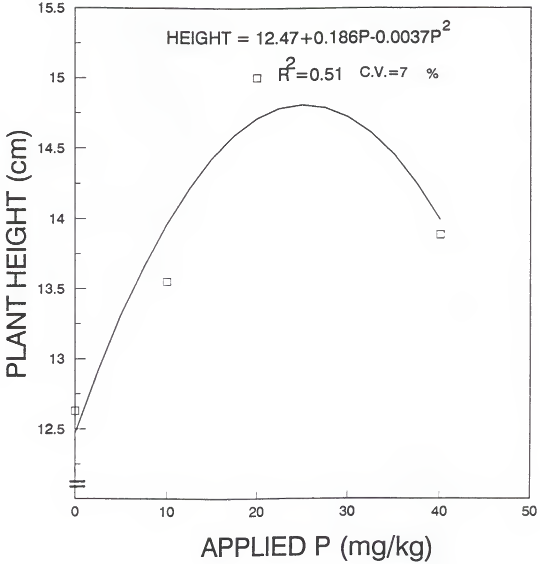

Fig. 6-1. Influence of soil by cultivar and P application on Porrillo 70 plant height in Horticultura soil from the P experiment in Honduras, 1983.

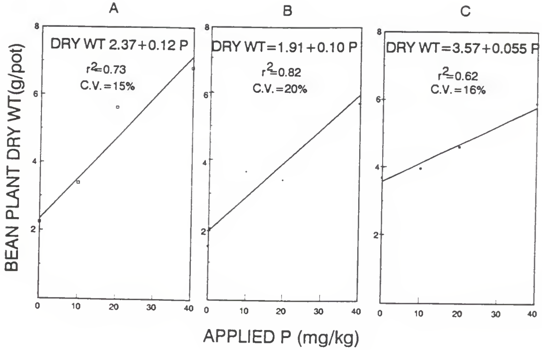

Fig. 6-2. Bean plant dry weight as influenced by soil, cultivar and P application. Fig. 6-2A shows Acacias 4 cultivar in Colindres soil; Fig. 6-2B shows Porrillo S. cultivar in Colindres soil and Fig. 6-2C shows Porrillo S. cultivar in Horticultura soil for the P experiment in Honduras, 1984.

Table 6-2. Analysis of variance of bean dry weight and total N content from the P experiment in Honduras in 1984.

Source of variation		Dry wt		Total N	
		MS	F	MS	F
Soil	1	5.35	**	12.3	NS
Cultivar	1	3.84	**	49.0	*
Soil*Cultivar	1	2.30	*	4.0	NS
Phosphorus	3	18.56	**	23.5	NS
Soil*P	3	10.25	**	28.5	NS
Cultivar*P	3	1.18	*	21.5	NS
Soil*Cultivar*P	3	5.15	**	16.2	NS
Error	48	0.42		11.3	
Total	63				
C.V. (%)		15		13	

*, ** significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

Effect of N Source

Combined N applied with 40 mg P kg^{-1} was compared to the combination of inoculated treatments with the same amount of P. Analysis of variance is presented in Table 6-3. Results are presented in Table 6-4 and Table 6-5.

Bean plant dry weight. Bean plant dry weight was influenced by the interaction of soil, cultivar, and N source (Table 6-4). In Colindres soil with the Acacias 4 cultivar, the inoculated treatment resulted in plant dry weight comparable to the combined N treatment. However, for Porrillo Sintetico cultivar in Colindres soil and for both cultivar in the Horticultura soil, the combined N treatment resulted in greater plant dry weight. This synergistic relationship between combined N and P has been attributed to N-induced increases in P absorption by the plant (Terman et al. 1977).

In three out of four cases inoculated treatments resulted in lower plant dry weight than did treatments with the combined N treatment; this reconfirms the tendency of inoculated beans to have lower plant dry weight than beans receiving combined N treatment.

Table 6-3. Analysis of variance of the effect of N source on dry weight and N content of bean plants with 40 mg P kg^{-1} from the P experiment in Honduras in 1984.

Source of variation	df	Dry wt MS	Dry wt F	N content MS	N content F
Soil	1	0.76	NS	22.8	NS
Cultivar	1	0.05	NS	357.8	**
Soil*Cultivar	1	0.02	NS	0.3	NS
N source	1	68.65	**	19.5	NS
Soil*N source	1	3.09	NS	205.0	**
Cultivar*N source	1	2.72	NS	166.5	**
Soil*Cult*N source	1	17.75	**	63.3	*
Error	24	0.83		14.6	
Total	31				
C.V. (%)		16		14	

*, ** Significant at 0.05 and 0.01 probability levels, respectively. NS=Not significant.

Table 6-4. Effect of the interaction of soil, cultivar and N source on bean plant dry weight from the P experiment in Honduras in 1984.

Cultivar	Nitrogen source	Dry wt	Contrast	C.V. (%)
		g pot^{-1}		
	Colindres soil			
Acacias 4	Inoculated	5.64		
	Combined N	5.87	N.S.	9
Porrillo S.	Inoculated	3.44		
	Combined N	7.82	**	10
	Horticultura soil			
Acacias 4	Inoculated	3.79		
	Combined N	8.25	**	20
Porrillo	Inoculated	4.66		
	Combined N	7.31	**	19

*, ** Significant at 0.05 and 0.01 probability levels, respectively. NS=Not significant.

Table 6-5. Effect of the interaction of soil, cultivar and N source on total N content of bean plants from the P experiment in Honduras in 1984.

Cultivar	Nitrogen source	Total N	Contrast	C.V. (%)
		g kg^{-1}		
	Colindres soil			
Acacias 4	Inoculated	24.0	**	18
	Combined N	38.0		
Porrillo S.	Inoculated	24.5	NS	15
	Combined N	23.8		
	Horticultura soil			
Acacias 4	Inoculated	30.0	NS	9
	Combined N	28.3		
Porrillo S.	Inoculated	20.0	*	11
	Combined N	25.3		

*, ** Significant at 0.05 and 0.01 probability levels, respectively. NS=Not significant.

Total N content. Bean plant total N content was affected by the interaction of soil, cultivar, and N source (Table 6-5). For Acacias 4 cultivar in Colindres soil and Porrillo S. cultivar in Horticultura soil, combined N resulted in more N in the plant than did the inoculant. This agrees with Ruschel and Reuszer (1973) who found that combined N increased plant dry weight and total N but decreased BNF. However, data for the Porrillo Sintetico cultivar in Colindres soil and Acacias 4 cultivar in Horticultura soil show that both the inoculated and combined N treatments resulted in similar N content in the plant.

Conclusions

1983

Plant dry weight was affected by soil and cultivar but not by P. Only plant height in the Horticultura soil was favored by the application of P, resulting in a quadratic response. Maximum height occurred when 25 mg P kg^{-1} were applied. Soils from this experiment were limed to pH near 7.0. The lack of response to applied P could have been the result of negative effect of liming, that may affect P availability.

1984

In the 1984 experiment, no lime was applied and the results were different. Phosphorus application induced a more dramatic effect on plant dry weight in Colindres soils (from 1.88 without P application to 6.24 g pot^{-1} with 40 mg P kg^{-1}) than in Horticultura (from 4.48 to 5.21 g pot^{-1}). This was expected since the amount of P in Horticultura soils is higher than that of Colindres (17 versus 6 mg P kg^{-1}). The recommended P-fertilization rate for beans in Central America is less than 15 mg P kg^{-1}. This recommendation was based on a series of field experiments (Salazar et al., 1978). However, in the 1984 experiment a linear response to applied P up to 40 mg P kg^{-1} was obtained for plant dry weight, indicating that maximum bean dry weight might be obtained with even higher P rates. The combination of N and P resulted in higher plant dry weight, demonstrating the importance of these two nutrients to bean growth. Combined N also resulted in higher N content in two out of four soil-cultivar combinations.

CHAPTER 7
EFFECT OF MOLYBDENUM, LIME AND N SOURCE ON BIOLOGICAL NITROGEN FIXATION AND BEAN GROWTH

Introduction

The importance of Mo in legume growth has long been recognized. Molybdenum is especially important for legumes dependent on N_2 for growth, since it is an essential constituent of nitrate reductase and nitrogenase which control the reduction of inorganic nitrate, and help to convert N_2 to NH_3. Thus Mo is one of the important keys to N_2 fixation by legumes.

Molybdenum concentrations in plants are highly variable. Plants may contain from less than 0.1 mg Mo kg^{-1} dry matter to more than 300 mg kg^{-1}. Molybdenum concentration varies according to the part of the plant; generally Mo is more abundant in leaves than stems. However, in leguminous plants such as beans, effective nodules have been reported to have higher concentrations of Mo than other parts of the plants (Jacob-Neto et al., 1988). The critical Mo concentration in alfalfa and red clover for full activity of N_2 fixation was 10 and 4.8 mg kg^{-1}, respectively. Much

lower levels in the shoots (0.5 mg Mo kg^{-1}) have been shown to be adequate. Franco and Munns (1981) found a high correlation between the Mo contents of nodules and stems.

Although Mo levels in different species vary, it is generally believed that crops respond to Mo treatment when this element is present in plants in amounts lower than 0.1 mg kg^{-1} (Kaytal and Randhawa, 1983; Ishizuka, 1981). Since Mo is intimately involved in N metabolism of plants, its deficiency resembles N deficiency.

The Mo source has been shown to influence the survival of rhizobia applied to _Macroptilium atropurpureum_ seed. Graham and Morales (1974) concluded that either molybdic oxide or ammonium molybdate can be used for seed pelleting as a source of Mo, this method being a practical means of applying this essential nutrient. On the other hand, they found that sodium molybdate cannot be used in contact with _Rhizobium_ (as a seed coat) because it is highly toxic to the bacteria. Sodium molybdate is widely used in the field as fertilizer applied to the soil; however, no report has been found that mentioned toxicity of this compound when used as soil fertilizer. Molybdenum fertilizer recommendations vary from 0.2 to 0.5 kg Mo ha^{-1}. However, in some cases applications up to 2 kg Mo ha^{-1} have been reported.

Forbes et al. (1986) found that pH treatments including application of 3,360 kg dolomitic lime ha^{-1} and 840 kg S ha^{-1} influenced soybean yields as much as did Mo applications.

Highest yields were obtained on limed plots. The combination of lime and Mo, especially the highest rate of Mo (0.8 kg ha^{-1}), produced excess Mo content in the leaves and seeds in a range known to cause molybdenosis in livestock.

Little information relative to the Mo effect and its interaction with lime and combined N on beans was found; most of the literature comes from experiments conducted under extremely acid soil conditions in Brazil. Several experiments failed to show a Mo response because of other limiting factors. In Brazil, Ruschel and Eira (1969) obtained no Mo benefit in soybean until Mn toxiticy was eliminated. The objective of these experiments was to determine the effect of Mo and its interaction with lime and N on BNF and bean growth under Honduran soil conditions.

Materials and Methods

1983 Experiment

Soils collected from Colindres and Horticultura sites were air-dried, pulverized and passed through a sieve. Ten days prior to planting, lime was applied at two levels --zero and the lime required to increase pH to 6.5 for each soil [2.14 and 2.20 cmol ½ {$Ca(OH)_2$} kg^{-1} for Colindres and Horticultura soils, respectively]. Water was added immediately after applying the lime and the soil was mixed once daily for 10 d to ensure a homogeneous contact between the lime and the soil.

A 3-cm layer of washed gravel covered with filter paper was placed on the bottom of plastic pots. Two kilograms of soil were added to each pot. A nutrient solution containing all nutrients except Mo, Ca, and N was applied (Diaz-Romeu and Hunter, 1978). Five treatments --0, 0.06, 0.13, 0.25 and 0.5 mg Mo kg^{-1} using reagent grade sodium molybdate (39% Mo) as the source-- were included. Selected soil characteristics prior to treatments are presented in Table 3-2.

Eight grams of peat inoculant per 100 g of seeds were used with 2.5 mL gum arabic to coat the bean seeds. The procedure used was the one recommended by NifTAL (1980). Five air-dried seeds of Acacias 4 and Porrillo 70 cultivars were planted in their respective pots. After 1 wk, seedlings were thinned to two per pot.

The experiment was analyzed as a completely randomized design with three replications. Plants were harvested at flowering stage (R6). Plant and nodule dry weights as well as height of plants were recorded. Statistical analyses were calculated using the Statistical Analysis System (SAS, 1987).

1984 Experiment

The experiment with Mo conducted in 1983 was repeated in 1984. Lime at the rates of 0 and 1.87 for Colindres and 1.92 cmol ½ $Ca(OH)_2$ kg^{-1} for Horticultura, respectively, were applied prior to planting. Materials and methods were similar except cultivar Porrillo 70 was changed to Porrillo

Sintetico due to the lack of Porrillo 70 seeds. Granular inoculant was used in this experiment at the rate of 0.12 g per pot. A treatment with combined N at the rate of 50 mg N kg^{-1} was included in order to compare this treatment and the inoculated treatment. Both treatments have the same amount of Mo (0.13 mg Mo kg^{-1}). Clay pots containing 2.6 kg soil per pot were used. Plant dry weight and total N content were recorded.

The soils were analyzed separately. Data were analyzed as a completely randomized design with four replications. Statistical analyses were calculated using the SAS program (SAS, 1987). The first analysis was calculated with all the treatments containing inoculant. A second statistical analysis was calculated to compare the treatment that included 0.13 mg Mo kg^{-1} with inoculant versus the treatment that contained that amount of Mo but with combined N instead of the inoculant.

Results and Discussion

1983

For some unknown reason, neither cultivar grew well in Colindres soil in the 1983 Mo experiment. Only data from Horticultura soil are presented. Ruschel and Reuszer (1973); Chavez et al. (1977); Trigoso and Fassbender (1973) reported no effect on plant dry weight or nodule weight when Mo was applied to leguminous plants including beans. Similarly, in

this experiment Mo did not affect any of the measured parameters. This agreed with results from the first pot experiment in 1982 (Chapter 4) where no response of bean plant dry weight to Mo was obtained. Lucas and Knezek (1972) mentioned that Mo deficiency may occur when the supply of this nutrient in soil is low, such as in the Inceptisols, especially in soils high in free Fe_2O_3. However, they also reported the lack of response of beans to Mo similar to the one found in this study. This deficiency may be accentuated in areas with moderate to heavy rainfall. An analysis of variance of this experiment is presented in Table 7-1. Results are presented in Table 7-2 and 7-3.

Plant dry weight. Plant dry weight was influenced by the main effects of lime and cultivar. The Porrillo 70 cultivar produced higher plant dry weight than Acacias 4 cultivar (Table 7-2). Lime favored plant dry weight as shown in Table 7-3.

Plant height. Plant height was also affected by lime and cultivar. Porrillo plants were taller than Acacias 4 plants (Table 7-2). Again, liming increased plant height as is shown in Table 7-3. Molybdenum application did not affect plant height. Similar results have been reported by Bellintani-Neto and Lam-Sanchez (1974) in soybean, when amounts up to 0.2 mg Mo kg^{-1} were applied.

Table 7-1. Analysis of variance for plant dry weight and height of bean plants from the Mo experiment with Horticultura soil in Honduras, 1983.

Source of variation	df	Dry wt		Height	
		MS	F	MS	F
Cultivar	1	3.22	**	47.7	**
Lime	1	4.68	**	13.5	**
Molybdenum	4	0.39	NS	4.6	NS
Lime*Mo	4	0.74	NS	6.8	NS
Cult*Lime	1	0.39	NS	2.6	NS
Cult*Mo	4	0.22	NS	2.7	NS
Cult*Lime*Mo	4	0.49	NS	1.9	NS
Error	40	0.35		2.4	
C.V. (%)		28		14	

** Significant at the 0.01 probability level. NS=Not significant.

Table 7-2. Influence of cultivar on bean plant dry weight and plant height from the Mo experiment with Horticultura soil in Honduras in 1983.

Cultivar	Plant dry wt	Plant height
	--g pot^{-1}--	----cm ----
Acacias 4	1.9	11.3
Porrillo 70	2.3	13.1
$LSD_{0.05}$	0.3	0.7
C.V. (%)	28	14

Table 7-3. Influence of lime on bean plant dry weight and plant height from the Mo experiment with Honduras in 1983.

½$Ca(OH)_2$	Plant dry weight	Plant height
cmol kg^{-1}	---g pot^{-1}---	-----cm-----
0	1.8	11.7
2.20	2.4	12.7
$LSD_{0.05}$	0.3	1.0
C.V.(%)	28	14

1984.

Ruschel and Doberenier (1967) claimed that Mo was necessary for BNF, but in excess, it damaged the symbiosis more than plant development. In this experiment, mixed results were found with respect to response to Mo applications. Analyses of variance for the two soils are presented in Tables 7-4 and 7-5.

Colindres Soil

Plant dry weight. Plant dry weight was influenced by the interaction of cultivar, lime, and Mo levels. Acacias 4 cultivar was not affected by lime and Mo applications. However, Porrillo S. cultivar decreased in plant dry weight in response to Mo application in the limed soil (Fig. 7-1A). This agrees with the results of Trigoso and Fassbender (1973) who found in several leguminous plants a decrease in dry-matter production when Mo was applied. A possible explanation for this is that Mo in Colindres soil was sufficient for bean plants and the excess of Mo induced a toxic effect which reduced plant dry weight. In this particular case, the application of lime induced an excess of available Mo that could have caused a decrease in plant dry weight.

Total N content. Bean tissue N content was affected by cultivar; the Acacias 4 cultivar contained a higher quantity of total N than the Porrillo S. cultivar (Table 7-6).

Table 7-4. Analysis of variance of bean dry weight and total N content from the Mo experiment with Colindres soil in Honduras, 1984.

Source of variation	df	Dry wt MS	Dry wt F	Total N content MS	Total N content F
Cultivar (Cult)	1	0.20	NS	288.8	**
Lime	1	55.07	**	3.2	NS
Cult*Lime	1	0.07	NS	11.25	NS
Molybdenum (Mo)	4	4.43	**	29.4	NS
Cult*Mo	4	4.58	**	7.9	NS
Lime*Mo	4	2.97	**	6.7	NS
Cult*Lime*Mo	4	1.39	**	14.4	NS
Error	60	0.31		12.0	
C.V. (%)		9		13	

** Significant at the 0.01 probability level. NS=Not significant.

Table 7-5. Analysis of variance of bean dry weight and total N content from the Mo experiment with Horticultura soil in Honduras, 1984.

Source of variation	df	Dry wt MS	Dry wt F	Total N content MS	Total N content F
Cultivar (Cult)	1	10.19	**	28	NS
Lime	1	0.01	NS	16	NS
Cult*Lime	1	2.14	NS	22	NS
Molybdenum (Mo)	4	1.93	NS	27	NS
Cult*Mo	4	3.96	**	27	NS
Lime*Mo	4	3.99	**	26	NS
Cult*Lime*Mo	4	3.75	**	6	NS
Error	60	0.63		10.7	
C.V. (%)		16		13	

** Significant at the 0.01 probability level. NS=Not significant.

Table 7-6. Total plant N content as influenced by cultivar from the Mo experiment in Colindres soil in Honduras in 1984.

Cultivar	Total plant N
	------g kg^{-1})-------
Acacias 4	28
Porrillo S.	24
CONTRAST	
Acacias vs. Porrillo	**
C.V. (%)	13

** Significant at the 0.01 probability level. NS=Not significant.

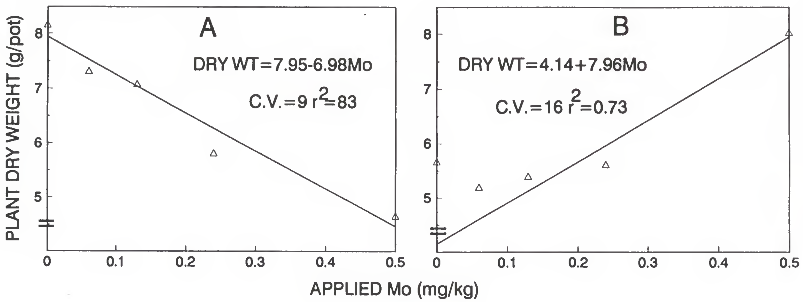

Fig. 7-1. Influence of the cultivar and Mo on bean plant dry weight from the Mo experiment in Honduras, 1984. Fig. 7-1A shows the effect of Mo on Porrillo S. cultivar from the limed Colindres soil. Fig. 7-1B shows the effect of Mo on the same cultivar from the limed Horticultura soil.

Differences in cultivars relative to their N content are related to N partitioning in the plants as reported by Hungria and Neves (1987).

Horticultura Soil

Plant dry weight. This parameter was affected by the interaction of cultivar, lime, and Mo applications. The Acacias 4 cultivar as in the Colindres soil did not respond to lime and Mo. However, Porrillo S. was favored by Mo application in the limed soil.(Fig. 7-1B). The Acacias 4 cultivar was not affected by Mo applications, neither was Porrillo S. cultivar when soil was not limed. Variable response to Mo at different soil pH values have been reported by Anderson and Mortvedt (1982) in soybeans in the U.S. One possible reason for inconsistency in the results are the differences in Mo content of seeds. This has been reported by Franco and Munns (1981) who found that Mo was 10 times higher in bean seeds produced in California in an Entisol than in Brazil in an Oxisol. They suggested that manipulation of plant genetic and environmental factors could be used to obtain the necessary Mo for bean growth in Mo-deficient soils. Franco and Day (1980) reported a response to Mo in beans when pH was between 5.2 to 5.8. They claimed that above pH 6.0 no response to Mo was obtained. This was explained by the increased availability of Mo when pH increases and also by increased absorption of Mo within

the plant (Lucas and Knezek, 1972). Soil pH of this experiment when soil was limed increased to pH 6.2. Liming soils to around pH 6.0 is high enough to supply Ca and to increase the availability of Mo (Adams, 1978).

Total N. Tissue N content was not affected by treatments. There was no difference in total N content in plants of the two cultivars.

Effect of N source

Molybdenum is closely related to N_2 fixation (Franco and Doberenier, 1967), and it has been reported to act synergistically with N. Treatments with 0.5 kg Mo ha^{-1} with combined N and inoculation were compared. An analysis of variance for this experiment is presented in Tables 7-7 and 7-8. Data are presented in Tables 7-9 through 7-12.

Colindres Soil

Bean plant dry weight. Bean plant dry weight was influenced by the interaction of lime and N (Table 7-9). The application of lime did not affect plant dry weight response to N source, thus both, the inoculated plants and the N-fertilized plants showed similar results. When no lime was applied the response in plant dry weight was higher for the combined N treatment than for the inoculated treatment.

Table 7-7. Analysis of variance of the effect of N source on bean plant dry weight and total N content from the Mo experiment with Colindres soil in Honduras in 1984.

Source of variation	df	Dry wt		Total N content	
		MS	F	MS	F
Cultivar (Cult)	1	0.74	NS	12.50	NS
Lime	1	1.53	NS	105.13	**
Cult*Lime	1	0.08	NS	8.00	NS
N	1	23.41	**	45.13	NS
Cult*N	1	21.11	**	84.50	*
Lime*N	1	13.22	**	78.13	*
Cult*Lime*N	1	0.71	NS	12.50	NS
Error	24	0.59		17	
C.V. (%)		11		16	

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

Table 7-8. Analysis of variance of the effect of N source on bean plant dry weight and total N content from the Mo experiment with Horticultura soil in Honduras in 1984.

Source of variation	df	Dry wt		Total N content	
		MS	F	MS	F
Cultivar (Cult)	1	0.39	NS	0.13	NS
Lime	1	13.84	**	144.50	**
Cult*Lime	1	5.18	NS	3.13	NS
N	1	39.22	**	55.13	**
Cult*N	1	0.05	NS	0.12	NS
Lime*N	1	7.36	*	10.12	NS
Cult*Lime*N	1	0.19	NS	8.00	NS
Error	24	1.44		8	
C.V. (%)		21		11	

*, ** Significant at the 0.05 and 0.01 probability levels, respectively; NS=Not significant.

Table 7-9. The interaction of lime and N source on bean plant dry weight and total N with 0.13 mg Mo kg^{-1} from the Mo experiment with the Colindres soil in Honduras, 1984.

N source	cmol ½$Ca(OH)_2$ kg^{-1}) 0	1.87	cmol ½$Ca(OH)_2$ kg^{-1}) 0	1.87
	Plant dry wt (g pot^{-1})		---- Total N----	
Inoculated	6.0	6.8	21	27
Combined N	9.0	7.3	27	28
CONTRAST				
Inoc vs. N	**	NS	**	NS
C.V (%)	18	13	18	15

** Significant at the 0.01 probability level. NS= Not significant.

Table 7-10. The interaction of cultivar and N source on bean plant dry weight and total N from the Mo experiment with the Colindres soil in Honduras, 1984.

N source	Cultivar Acacias 4	Porrillo	Cultivar Acacias 4	Porrillo
	Plant dry wt (g/pot)		-Total N (g kg^{-1})-	
Inoculated	5.8	7.1	24	25
Combined N	9.1	7.1	29	26
CONTRAST				
Inoc. vs. N	**	NS	**	NS
C.V. (%)	14	14	20	16

** Significant at the 0.01 probability level. NS= Not significant.

Table 7-11. The interaction of lime, and N source on bean plant dry weight and total N in plants with 0.13 mg Mo kg^{-1} from the Mo experiment with the Horticultura soil in Honduras, 1984.

N source	cmol ½$Ca(OH)_2$ kg^{-1}) 0	cmol ½$Ca(OH)_2$ kg^{-1}) 1.92	cmol ½$Ca(OH)_2$ kg^{-1}) 0	cmol ½$Ca(OH)_2$ kg^{-1}) 1.92
	Plant dry wt (g pot^{-1})		---- Total N-----	
Inoculated	4.5	4.8	21	26
Combined N	5.7	8.0	25	27
CONTRAST				
Inoc vs. N	*	**	**	NS
C.V (%)	19	22	10	11

** Significant at the 0.01 probability level. NS= Not significant.

Table 7-12. The interaction of cultivar and N source on bean plant dry weight and total N from the Mo experiment with the Horticultura soil in Honduras, 1984.

N source	Cultivar Acacias 4	Cultivar Porrillo S.	Cultivar Acacias 4	Cultivar Porrillo S.
	Plant dry wt (g/pot)		-Total N (g kg^{-1})-	
Inoculated	4.5	4.7	25	23
Combined N	6.7	7.0	26	26
CONTRAST				
Inoc. vs. N	**	**	NS	*
C.V. (%)	22	28	16	12

** Significant at the 0.01 probability level. NS= Not significant.

Bean dry weight was also influenced by the interaction of cultivar by N source. Bean dry weight was higher for Acacias 4 cultivar when combined N was applied (Table 7-10). when compared to inoculated plants. Porrillo S. cultivar was not affected by N source treatments.

<u>Total N content</u>. Nitrogen in plant tissue was affected by the interaction of lime and N source. When lime was applied both N treatments showed similar response (Table 7-9). Liming this soil favored BNF, possibly by increasing pH to 6.2 which resulted in higher N content in plant tissue. However, in the unlimed soil, plants from the combined N treatment showed higher N content in plant tissue when compared to the N content in inoculated plants.

Nitrogen in plant tissue was also affected by cultivar and N source (Table 7-10). Nitrogen content was higher in Acacias 4 plants when they were fertilized with combined N compared to the inoculated plants. On the other hand, Porrillo did not respond to N-source treatments.

<u>Horticultura Soil</u>

<u>Plant dry weight</u>. In the Horticultura soil the response to N source was lower than in the Colindres soil. Plant dry weight was affected by lime and N source (Table 7-11). The application of lime on bean plants fertilized with combined N resulted in a higher plant dry weight than when plants were inoculated. This synergistic interaction of lime with

combined N could be explained by the fact that lime increases the availability of mineral N and neutralizes toxic elements favoring nodulation and N_2 fixation (Freire, 1984) and also favoring plant growth. On the other hand, Porrillo S. cultivar showed similar results when N was applied as when inoculant was applied.

Plant dry weight was also affected by the interaction of cultivar and N source (Table 7-12). In this case, the Acacias 4 cultivar had higher plant dry weight when combined N was used than plants from the inoculated treatment. Acacias 4 cultivar did not respond to N treatments.

Plant total N. Bean plant total N content was influenced by the interaction of lime and N source (Table 7-11). When lime was applied, no differences in N were found in the inoculated treatment versus the combined N treatment. Liming the soil favored N_2 fixation possibly by increasing the pH to 6.2 which resulted in a N content in inoculated plants comparable to that in plants fertilized with combined N. However, when the soil was not limed, the combined N treatment resulted in more N in bean plants.

Nitrogen content in plant tissue was affected by the interaction of cultivar and N source (Table 7-12). No differences were found for Acacias 4 cultivar when treated with combined N or inoculation. However, Porrillo S. showed higher N content when combined N was applied compared to the inoculated plants.

Conclusions

The lack of response to Mo in 1983, and the differences in soil, cultivar, and lime interactions with Mo applications make specific conclusions difficult to draw. However, in most of the cases Mo applications did not increase bean growth or total N in bean plants. The reasons for this lack of response may be related to the soil characteristics. It is possible that Colindres soil is not deficient in this element. Another reason for the lack of response could be that the bean seed Mo content was sufficient to support plant growth. For these soils without Mo deficiency, no Mo should be applied. Application of lime favored bean-plant growth and plant N content in both soils. The Porrillo S. cultivar showed higher plant dry weight and height than Acacias 4 cultivar.

The application of combined N resulted in higher bean plant dry weight; this effect was more noticeable in Acacias 4 cultivar than Porrillo S. cultivar.

CHAPTER 8
EFFECT OF LIME, P, MO, AND N SOURCE ON BIOLOGICAL NITROGEN FIXATION AND BEAN YIELD IN FIELD EXPERIMENTS

Introduction

Several investigators have reported a positive response to Rhizobium inoculation of beans. Most of the work with rhizobia has been done in trials with plant growth in sand or solution culture in glasshouse experiments under controlled conditions. However, in field experiments response has varied greatly (Graham, 1981; Graham and Halliday, 1977). Lack of response to inoculation in beans is rather common (Huntington et al., 1986; Chavez, et al., 1977). There are many factors that influence the response of beans to inoculation in the field. Response to inoculation has been reported often to be site specific (Wynne et al., 1987; Halliday, 1984). It is important to have this in mind when recommendations are given.

The field environment is a complicated system where many factors are not as easily controlled as in glasshouse experiments. Thus, it is important to evaluate the effects

of soil factors on BNF under field conditions.

There are few field studies with rhizobia but information available is sufficient to indicate that differences in yield result from a pH, Ca (Andrew 1977), and/or phosphate response [Jones (1974); Ozanne et al. (1969); Munns and Fox (1977); Munns et al. (1977), Spain et al. (1975); Munns and Mosse (1978); Dhingra et al. (1988)]. In some cases response to lime has been extremely variable.

Response to Mo and other micronutrients has also been variable. Response to any soil factor will depend on the legume species and even on the cultivar. Black beans have been reported to have a higher capacity to fix N_2 than red-colored beans. Growth habit also has a large influence on the potential of the plants to fix N_2. Several authors have reported that indeterminate or semideterminate-growth-habit cultivars have a greater capacity to fix N_2 (Rennie and Kemp, 1983b; Graham and Rosas, 1977) than determinate types.

Three field experiments were conducted at the Escuela Agricola Panamericana to substantiate results obtained in pot experiments, and to determine the effect of the interactions of the nutrients studied on bean yield and BNF in a field environment.

Materials and Methods

1982 Experiment

The objective of this experiment was to determine the effect of lime, P, and Mo on beans that were inoculated, uninoculated, and fertilized with N. The experiment was located in two plots of the Horticultura site; plot 21 was planted on 27 August and plot 46 was sown on 30 August. Soil from plot 21 had been used for several glasshouse studies (Chapters 5, 6, and 7). Both soils have been classified as Vertic Haplustalfs (Anon., 1989). Chemical characteristics of these soils are presented in Table 3-2.

Lime rates [0, 1823, and 2735 kg $Ca(OH)_2$ ha^{-1}] corresponding to 0, 1, and 1.5 times the recommended amount of lime to increase soil pH to 6.5 (Adams and Evans, 1962) were broadcast and incorporated with hoes 3 wk before planting. Molybdenum was applied at the rate of 0 and 1.2 kg Mo ha^{-1} as sodium molybdate; P was applied at 0 and 80 kg ha^{-1} as triple superphosphate (46% P_2O_5). A mixture of three different sources (see Chap. 4) of _R_. _leguminosarum_ bvar. _phaseoli_ in granular form at the recommended rate of 9.2 kg ha^{-1} was applied to all treatments. Two more treatments were included. The first one was a control plot without inoculant, P, or Mo. A second one, called "complete" included P (80 kg ha^{-1}), Mo (0.5 kg ha^{-1}) and N (100 kg ha^{-1}) using urea (46%N); this latter treatment did not include

inoculant. All fertilizers were applied before planting. Plots measured 6 by 3 m. Fifty centimeters were left between rows. Plots consisted of six rows planted with Acacias 4 cultivar with 8 cm between each seed to give a plant population of 250,000 ha^{-1}. The four central rows were harvested. Five plants were collected at 50% flowering from the adjacent rows to determine plant dry weight, total foliar N, plant color, plant vigor, and nodule and root weights.

The experimental design was a split plot where site was the main plot and treatments were arranged in a randomized complete-block design with four replications at each site. Two analyses of variance were calculated using the SAS program (SAS, 1987). The first one included the 12 inoculated treatments. The second was performed comparing the control treatment with and without inoculant. The complete treatment with inoculant was compared to the complete treatment with combined N.

1983 Experiment

This experiment was conducted in order to determine the effect of lime, P, Mo, and combined N on BNF and bean yields, as well as to determine if interactions exist between these nutrients. Field studies were conducted during September 1983 in the Colindres and Horticultura sites. The

former was classified as Typic Ustifluvent. The latter soil has been classified as Vertic Haplustalf (see Chapter 3).

Maize was grown in the plots for 13 wk before the beans were planted. The objective of this was to allow maize to remove some of the nutrients creating the typical low soil fertility conditions encountered in relay planted beans. After maize plants were removed and before beans were planted, paraquat was applied with a wick applicator at the rate of 2 L ha^{-1}. Weeds were controlled after bean emergence with hoes. Slugs were controlled by applying a homemade bait containing carbaryl and methaldehyde at the edges of the experimental plots.

A broadcast application of lime was incorporated 2 wk prior to planting. Lime was applied at the rates of zero and the amount recommended to increase pH to 6.5 (1768 and 1823 kg $Ca(OH)_2$ ha^{-1} for Colindres and Horticultura soils, respectively). Fertilizer was applied in each trench according to the treatments. A general application of Mg at 24 kg ha^{-1} as magnesium sulfate was applied. Nitrogen was applied as urea (46%N) at the rates of zero and 100 kg N ha^{-1}. Phosphorus rates were 0 and 80 kg P ha^{-1} from triple superphosphate (46% P_2O_5). Molybdenum was applied at the rates of 0 and 0.5 kg ha^{-1} as sodium molybdate (39% Mo); sodium molybdate used in this experiment was reagent quality. In order to assure a homogeneous application of Mo in plots where Mo was the only fertilizer, this compound was

first mixed with a small amount of soil collected from the same plot and the mixture was then spread. A granular mixture of the three strains (exotic and native) of Rhizobium inoculants was applied at the rate of 9.2 kg ha^{-1} next to the seeds, then covered with soil.

The experimental design was a stripped factorial design. Treatments were replicated three times. Cultivars were stripped across the plots. Plots of 4 by 4 m were planted. Each experimental unit consisted of four rows of each of the two cultivars, Acacias 4 and Porrillo Sintetico. Distance between rows was 50 cm and between plants was 8 cm. The bean population was calculated to be 250,000 plants ha^{-1}. Four central rows consisting of two rows of each cultivar were harvested. Five plants were collected at flowering stage (R6) from the adjacent rows. Plants were ground and dried at 70 ^{0}C until constant weight was obtained. Plant dry weight and grain yield (at 14% moisture) were recorded. Statistical analyses were calculated using the SAS program (SAS, 1987).

1984 Experiment

The 1983 experiment was repeated in September 1984 in plots adjacent to the 1983 trials. Treatments were the same except that granular inoculant was applied at 92 kg ha^{-1}, an amount 10 times higher than the recommended rate.

Lime was applied at the rates of 0 and the amount recommended of lime to increase pH to 6.5 [1546 and 1593 kg $Ca(OH)_2$ ha^{-1} for Colindres and Horticultura, respectively] 2 wk prior to planting. Other fertilizers were applied below the bean seeds at planting time. Phosphorus was applied at the rates of 0 and 80 kg P ha^{-1} using triple superphosphate (46% P_2O_5). Molybdenum was applied at the rates of 0 and 0.5 kg ha^{-1}, using reagent quality sodium molybdate (39% Mo). Treatments of N --0 and 100 kg N ha^{-1}, from urea-- were included in the experiment to compare with the inoculated treatment. Treatments were replicated four times in Colindres and three times in Horticultura site. The experimental design as well as data recorded were similar to the 1983 field experiment. Five plants with their roots were collected at flowering stage (R6). Nodules were washed, separated from the plants, and then compared with nodules from plants of the control (without N or Rhizobium) treatment using the following rating:

Score †		Description
1	Poor	Below 50% of control
2	Below average	51 to 80% of control
3	Average level	81 to 120% of control
4	Higher than average	121 to 150% of control
5	Highly superior	Above 150% of control

† (Rosas and Bliss, 1984).

Plants were dried at 70 ^{0}C until constant weight was obtained. Bean plant dry weight, total N content, nodule score (Rosas and Bliss, 1984) and grain yield (at 14% moisture) were recorded. Statistical analyses were calculated with the SAS program (SAS, 1987). A first analysis was calculated using all treatments. A second analysis was calculated to disclose interactions of studied factors.

Results and Discussion

1982 Experiment

Parameters evaluated in this experiment included dry weight of plants, nodules and roots; height, vigor, color and total N at flowering stage (R6); and grain yield at 14% moisture. None of the characteristics measured in this experiment showed positive response to treatments, except site. Only bean yield analysis of variance is shown in Table 8-1.

Even though the two sites studied in this experiment were less than 1 km apart and they have been classified as Vertic Haplustalfs, yield differences among sites were noted. Both yields were lower than the average Honduran yield of 600 kg ha^{-1}. Plot 46 produced lower yield than plot 21 (225 versus 350 kg ha^{-1}). Plot 46 had more problems with water logging and in general is a soil with lower potential

for bean growth. One of the reasons for low yield was that these soils have problems with weeds, most notably Cyperus rotundus, a cyperaceous weed that has plagued the soils from Horticultura site. Crops are also severely affected by diseases and insects. The differences between the two sites were mostly due to differences in soil characteristics and management.

A comparison of the two control treatments (without P, lime, and Mo) with and without inoculation showed that only nodule dry weight was affected by inoculation (Table 8-2). Inoculated plants had higher nodule dry weight than non-inoculated. This result agrees with that found by Mangual-Crespo et al. (1987) who found that Rhizobium inoculation positively influences the degree of nodulation. This increase in nodulation implies that Rhizobium strains from the inoculant were able to infect the bean plants. However, the N content of the plants did not differ between treatments. This could mean that even if strains are infective they do not form a successful symbiosis with bean plants. This was observed in one of the pot experiments that was discarded due to a similar situation; plants were nodulated but deficient in N as determined by visual observations (Chapter 5); this situation is also rather common in the literature (Huntington et al., 1986; Jimenez and Villalobos, 1980). Photosynthate distribution has been cited as one of the reasons why bean cultivars do not fix

enough N_2 for bean growth (Adams et al., 1978). Another reason mentioned in the literature is also the variability of this photosynthate distribution even among cultivars.

The comparisons of the complete treatments both of which included P, lime, and Mo are presented in Table 8-3. Plant dry weight was affected by the N source; when combined N was applied, plant dry weight was higher than when inoculant was applied. Nodule dry weight was also affected by N source; nodulation was superior in the inoculated treatment when compared with the combined N. This is commonly ascribed in the literature to the inhibition of nodulation by the presence of N. Bergersen (1982) suggested that BNF is a survival mechanism that increases only when N is lacking. Inhibition of nodulation due to combined N was commonly found in pot experiments in this research, and it has been reported by several authors (Awonaike et al., 1980; Evans, 1982; Graham, 1981).

Total N in plant tissues fertilized with combined N were higher than in the inoculated plants. Similar response was obtained with yields, plants fertilized with combined N treatment showed higher yield than inoculated plants. However these increases were not significant at the 0.05 probability level. Overall, data obtained in this experiment shows that inoculant do not seem to do well for beans. All parameters consistently favor combined N over inoculant.

Table 8-1. Analysis of variance for yield obtained from the field experiment in two Horticultura soils in Honduras, 1982.

Source of variation	df	Bean yield	
		MS	F
Site	1	374970	**
Rep (site) †	6	34317	
Lime	2	11317	NS
Molybdenum (Mo)	1	3722	NS
Lime*Mo	2	3231	NS
Phosphorus (P)	1	979	NS
Lime*P	2	8714	NS
Mo*P	1	1416	NS
Lime*Mo*P	2	159	NS
Site*Lime	2	1936	NS
Site*Mo	1	3593	NS
Site*P	1	13306	NS
Error	73	4215	NS

** Significant at the 0.01 probability level. NS=Not significant.
† Rep=replicate

Table 8-2. Comparison of the effect of control treatment with and without inoculant on several parameters. Honduras, 1982.

Parameter	Inoculated	Uninoculated	Contrast (Prob.)
Dry wt (g/5 plts)	18	18	0.86 NS
Nod wt (mg/5 plts)	350	263	0.02 **
Root wt (g/5 plts)	4	4	0.54 NS
Height (cm)	21.5	20.7	0.63 NS
Vigor †	2	2	0.73 NS
Color ‡	2	2	0.19 NS
Total N (g kg^{-1})	26	24	0.92 NS
Yield (kg ha^{-1})	292	300	0.80 NS

† Vigor scale ranges from 1 to 5, where 1=best and 5=worst.
‡ Color scale ranges from 1 to 4, where 1=best and 4=worst.
** Significant at the 0.01 probability level. NS=Not significant.

Table 8-3. Comparison of the effect of complete treatments with inoculant versus combined N on various parameters. Honduras, 1982.

Parameter	Inoculated	Combined N	Contrast (Prob.)
Dry wt (g/5 plts)	20	35	0.05 **
Nod wt (mg/5 plts)	288	175	0.03 *
Root wt (g/5 plts)	4.1	5.8	0.12 NS
Height (cm)	21	24	0.31 NS
Vigor †	2	1	0.20 NS
Color ‡	2	1	0.35 NS
Total N (g kg^{-1})	34	38	0.09 NS
Yield (kg ha^{-1})	267	374	0.06 NS

† Vigor scale ranges from 1 to 5, where 1=best and 5=worst.
‡ Color scale ranges from 1 to 4, where 1=best and 4=worst.
*,** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

1983 Experiment

Only the Colindres trial was harvested. The Horticultura trial was lost due to excess moisture though it was replanted three times. The Horticultura site has a history of water logging. Beans are extremely sensitive to water logging (Espinosa-Victoria et al., 1985; Freire, 1984). An analysis of variance for the Colindres soil is presented in Table 8-4. Data of plant dry weight and height are presented in Table 8-5 and Table 8-6. Nodule weight was not measured but visual observations indicated that combined N reduced the number of nodules. Also Acacias 4 cultivar produced more nodule weight than Porrillo S. cultivar.

Plant dry weight. Plant dry weight was influenced by the interaction of Mo and N source (Table 8-5) and by the main effect of P. When Mo was not applied, the inoculated treatment was inferior to the 0 N and 100 kg N ha^{-1}. No difference was found in bean plant dry weight for the three N treatments. Application of P increased plant dry weight. When P was applied at 80 kg ha^{-1}, plant dry weight increased to 18 g/5plants compared to 12 g/5 plants without P fertilizer. Phosphorus is commonly cited in the Central American literature as one of the most important nutrients for the majority of crops (Bazan, 1975; Stryker, 1977; Fassbender and Bornemiza, 1987). In these soils even if P has been applied intensively, positive responses to P are often obtained. This was found in the 1984 pot experiment

where a linear dry-weight response to P occurred for both the Colindres and Horticultura soils.

Height. Height was influenced by the interaction of cultivar and P (Table 8-6). The application of P produced taller plants in Acacias cultivar. Difference was found between no application of P versus 80 kg P ha^{-1} in Porrillo S. cultivar. The application of P resulted in a more vigorous plant in Acacias cultivar than in Porrillo S. Differences in P requirements of cultivars has been reported by Cassman et al. (1980). However, foliar samples of these two cultivars showed similar amounts of P content.

Total N. Plant total N was affected by P and N source. Total N was higher when P was applied than when no P was applied. Phosphorus increased N content in chickpea plants has been determined by Parihar and Tripathi (1989). The synergism of P and N has been reported by several authors (Cassman et al., 1980; Adams, 1980; Summer and Farina, 1986). The application of combined N produced higher amounts of total N in the plants fertilized with N than in plants of the control and inoculated treatments (35 versus 33 and 32 g N kg^{-1}, respectively). This agrees with what was found in most of the cases in the pot experiments, and also reported in the literature (Ruschel and Reuszer, 1973; Ruschel et al., 1982).

Table 8-4. Analysis of variance for field experiment in Colindres soil in Honduras in 1983.

Source of variation	df	Dry wt	Bean yield	Height	Total N
		---------- Probability	-----------	-----------	-----------
Replicate	2	NS	NS	**	*
Cultivar (C)	1	NS	NS	NS	NS
Lime (L)	1	NS	NS	NS	NS
Phosphorus (P)	1	**	NS	**	**
L*P	1	NS	NS	NS	NS
Molybdenum (Mo)	1	NS	NS	NS	NS
L*Mo	1	NS	NS	NS	NS
Mo*P	1	NS	NS	NS	NS
L*Mo*P	1	NS	NS	NS	NS
N	2	**	**	NS	**
L*N	2	NS	NS	NS	NS
P*N	2	NS	NS	NS	NS
L*P*N	2	NS	NS	NS	NS
Mo*N	2	**	NS	NS	NS
L*Mo*N	2	NS	NS	NS	NS
Mo*P*N	2	NS	NS	NS	NS
L*MO*P*N	2	NS	NS	NS	NS
C*L	1	NS	NS	NS	NS
C*P	1	NS	NS	*	NS
C*Mo	1	NS	NS	NS	NS
C*N	2	NS	NS	NS	NS
C*L*P	1	NS	NS	NS	NS
C*L*Mo	1	NS	NS	NS	NS
C*L*N	2	NS	NS	NS	NS
C*Mo*P	1	NS	NS	NS	NS
C*P*N	2	NS	NS	NS	NS
C*Mo*N	2	NS	NS	NS	NS
C*L*Mo*P	1	NS	NS	NS	NS
C*L*P*N	2	NS	NS	NS	NS
C*Mo*P*N	2	NS	NS	NS	NS
C*L*Mo*N	2	NS	NS	NS	NS
C*L*Mo*P*N	2	NS	NS	NS	NS

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

Table 8-5. Influence of Mo and N sources on plant dry weight for Colindres soil in Honduras, 1983.

N applied	Mo applied, kg ha^{-1}	
	0	0.5
kg ha^{-1}	Plant dry weight (g/5 plts)	
0	17	14
100 †	17	14
none+inoc.	12	15
CONTRASTS		
0 N vs. Inoc.	**	NS
100 N vs. Inoc.	**	NS
C.V.(%)	39	32

† Source of N was urea (46%N). N is expressed in kg ha^{-1}
** Significant at the 0.01 probability level. NS=Not significant.

Table 8-6. Effect of fertilizer P and cultivar on plant height from the Colindres field experiment in Honduras, 1983.

P	Cultivar	
	Acacias 4	Porrillo S.
kg ha^{-1}	------Plant height (cm)------	
0	37	31
80	43	34
CONTRAST		
0 P vs. 80 P	**	*
C.V. (%)	16	17

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

Table 8-7. Analysis of variance for the field experiment in Colindres site in Honduras, in 1984.

Source of variation	df	Dry wt	Bean yield	Total N	Nod.
		---------Probability	------------		
Replicate	3	NS	NS	NS	NS
Cultivar (C)	1	NS	NS	NS	NS
Lime (L)	1	**	**	NS	NS
Phosphorus(P)	1	**	**	**	NS
L*P	1	NS	**	NS	NS
Molybdenum (Mo)	1	NS	*	NS	NS
L*Mo	1	**	*	*	NS
Mo*P	1	NS	NS	NS	NS
L*Mo*P	1	NS	**	NS	NS
N	2	**	**	**	**
L*N	2	NS	*	NS	NS
P*N	2	**	NS	NS	NS
L*P*N	2	*	NS	*	NS
Mo*N	2	**	*	NS	NS
L*Mo*N	2	**	NS	NS	NS
Mo*P*N	2	**	**	NS	NS
L*Mo*P*N	2	**	**	NS	NS
C*L	1	NS	NS	NS	NS
C*P	1	**	**	NS	NS
C*Mo	1	**	**	NS	NS
C*N	2	NS	**	NS	NS
C*L*P	1	NS	NS	NS	*
C*L*Mo	1	NS	*	NS	NS
C*L*N	2	NS	*	NS	NS
C*Mo*P	1	NS	NS	NS	NS
C*P*N	2	NS	NS	NS	NS
C*Mo*N	2	**	**	NS	NS
C*L*Mo*P	1	NS	NS	NS	NS
C*L*P*N	2	NS	NS	NS	NS
C*Mo*P*N	2	*	*	NS	NS
C*L*Mo*N	2	**	*	NS	**
C*L*Mo*P*N	2	NS	**	NS	NS

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

Bean yield. Bean yield was influenced by N sources. Combined N application resulted in higher yield than the control and the inoculated (1980 versus 1826 versus 1672 kg ha^{-1}, respectively) treatments. This is commonly cited in the literature, especially in tropical countries where beans are claimed to be poor N_2-fixers and respond to N fertilization (Graham and Halliday, 1977; Huntington et al., 1986; Cuautle, 1979; Vincent, 1974).

1984 Experiment

An analysis of variance for this experiment is presented in Table 8-7 for Colindres soil, and Table 8-16 for Horticultura soil. Results are presented in Tables 8-8 to Table 8-25.

Colindres Soil

Plant dry weight. Plant dry weight was influenced by the interaction of cultivar, lime, Mo and N source. In Acacias 4 cultivar (Table 8-8) when lime was not applied, the inoculated treatment resulted in similar dry weight to the control treatment with or without Mo; there was no Mo influence on plant dry weight. The application of combined N without lime and Mo produced similar results to the control and the inoculated treatments. This could mean that other factors were limiting bean plant dry weight. However, the combination of combined N and Mo but without lime produced

50 % more plant dry weight than the inoculated treatment. The application of Mo seemed to act synergistically with combined N to produce a higher bean dry weight. This agrees with what was found in pot experiments with Mo; when N and Mo were applied a higher plant dry weight was obtained relative to the inoculated treatment. Parker and Harris (1977) in soybeans, reported a greater response when both nutrients were applied than when each nutrient was applied singly.

Lime application without Mo resulted in more bean plant dry weight for the control than for the inoculated treatment. Again, other factors may have negatively influenced the bacteria resulting in lower plant dry weight. The addition of combined N and lime without Mo resulted in superior plant dry weight relative to the control and inoculated treatment. The combination of lime, Mo, and combined N resulted in higher plant dry weight than the other two N treatments. No differences were detected between the control and inoculated plants for the combination of lime and Mo. These data suggest that lime interacted synergistically with combined N and Mo resulting in higher plant dry weight. This positive response to lime was not observed in pot experiments with Colindres soil where lime depressed bean plant dry weight. Depression of legume growth was found to be temporal by Munns and Fox (1976) who warned that short-term trials and especially pot trials which

restrict roots to treated soil, should be used with caution to predict yield responses. Adverse effects of lime may sometimes have less practical importance than short-term experiments would indicate.

In Porrillo S. cultivar similar bean plant dry weight was obtained for the three N treatments (control, combined N treatment and inoculated) when no lime and no Mo were applied. The application of Mo resulted in a higher plant dry weight for the control than the inoculated. It appears that Mo was toxic to the Rhizobium of the inoculated treatment, as was noted in the 1984 Mo pot experiment and also has been reported by Franco and Doberenier (1967). Application of combined N and Mo resulted in higher plant dry weight.

The application of lime without or with Mo resulted in similar response of the inoculated and the control. However, combined N and lime produced higher plant dry weight especially when no Mo was applied. The lime x N interaction was also observed in the 1984 lime experiment (Chapter 5).

Bean plant dry weight was also influenced by the interaction of cultivar, P, Mo, and N source in Colindres soil (Table 8-9). For the Acacias 4 cultivar, the inoculated treatment without combined N, P, and Mo produced a bean dry weight similar to the control. However, when N was added, higher bean plant dry weight resulted.

Table 8-8. Influence of cultivar, lime, Mo, and N source on bean dry weight from the Colindres site in Honduras, 1984.

N applied	Lime (kg ha^{-1})			
	0		1546	
	Mo (kg ha^{-1})		Mo (kg ha^{-1})	
	0	0.5	0	0.5
kg ha^{-1}	----Bean plant dry wt (g/5 plts)----			
	Cultivar: Acacias 4			
0	40	36	39	38
100	37	65	58	49
none+inoc.	32	27	31	39
CONTRASTS				
0 N vs. Inoc.	NS	NS	*	NS
100 N vs. Inoc.	NS	**	**	*
C.V. (%)	24	32	19	24
	Cultivar: Porrillo Sintetico			
0	30	42	39	40
100	46	50	60	48
none+inoc.	40	24	43	34
CONTRASTS				
0 N vs. Inoc.	NS	*	NS	NS
100 N vs. Inoc.	NS	**	**	**
C.V. (%)	38	42	26	28

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

The application of Mo to inoculated treatment resulted in lower plant dry weight than the control. Again, apparently, Mo was toxic to the inoculated plants. However, with the application of combined N plants appeared to overcome the negative effect of Mo and showed a higher plant dry weight. Plants fertilized with combined N are more vigorous and hence, have a greater capability to absorb nutrients. When P was applied, the inoculated treatment was still lower than the control, and once more the combined-N treatment produced a higher plant dry weight. When the combination of Mo and P was applied, the inoculated treatment was similar to the control.

When no P was applied, the Porrillo S. plants from the inoculated treatment were similar to those from the control treatment with and without Mo. However, when P was applied, without Mo, the three N treatments (control, combined-N and inoculated) resulted in a similar plant dry weight. The application of Mo and P resulted in similar plant dry weight for the control and inoculated treatments. Application of combined N treatment, P and Mo resulted in higher plant dry weight than the other two. A synergistic effect of P with Mo and N has been reported (Sumner and Farina, 1986; Pal et al., 1989; Katyal and Randhawa, 1983). The positive interaction of P and Mo on Mo uptake by plants has been related to the plant's metabolic processes involving these

two ions though the exact mechanism is still not known (Adams, 1980).

Total plant N. Total plant N content was influenced by the interaction of lime, P, and N source (Table 8-10). When P was not applied there was no response to lime or to combined N. The three N treatments resulted in similar N content in the plant. When P was applied, only the combined-N treatment without lime resulted in higher N content in the plant. The application of lime resulted in similar results for the three N treatments. This synergistic effect of P and combined N on plant total N has been reported in Chapter 7, and by other authors such as Sumner and Farina, 1986; Adams, 1980.

Total plant N was also influenced by the interaction of lime with Mo (Table 8-11). When Mo was not applied but lime was applied, total N in the plant was higher than when no lime was applied. Lime enhanced total N content in plants. The application of Mo did not have any effect on total N content. This does not agree with the results of Parker and Harris (1977) who found that soybean-leaf N increased when Mo was applied.

Table 8-9. Influence of the interaction of cultivar, P, Mo, and N source on bean-plant dry weight from the Colindres site in Honduras, 1984.

N applied	P (kg ha^{-1})			
	0		80	
	Mo (kg ha^{-1})		Mo (kg ha^{-1})	
	0	0.5	0	0.5
kg ha^{-1}	---------Plant dry wt (g/5plts)--------			
	Cultivar: Acacias 4			
0	35	37	44	37
100	43	46	51	69
none+inoc.	32	28	31	38
CONTRASTS				
0 N vs. Inoc.	NS	*	*	NS
100 N vs. Inoc.	**	**	**	**
C.V. (%)	22	18	27	30
	Cultivar: Porrillo S.			
0	25	21	43	41
100	45	49	60	68
none+inoc.	33	24	51	34
CONTRASTS				
0 N vs. Inoc.	NS	NS	NS	NS
100 N vs. Inoc.	**	**	NS	**
C.V. (%)	31	20	24	19

** Significant at the 0.01 probability level. NS=Not significant.

Table 8-10. Effect of lime, P, and N source on total N in bean plants from the field experiment at the Colindres site in Honduras, 1984.

N applied	P (kg ha^{-1})			
	0		80	
	Lime (kg ha^{-1})		Lime (kg ha^{-1})	
	0	1546	0	1546
kg ha^{-1}	-------Bean plant total N (g kg^{-1})--------			
0	27	28	25	28
100	29	31	29	28
None+inoc.	28	30	26	27
CONTRASTS				
0 N vs. Inoc.	NS	NS	NS	NS
100 N vs. Inoc.	NS	NS	*	NS
C.V.(%)	13	11	13	9

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

Table 8-11. Effect of the interaction of lime and Mo on total N in bean plants for the field experiment from Colindres site in Honduras, 1984.

Lime	Mo (kg ha^{-1})	
	0	0.5
kg ha^{-1}	-Bean plant total N (g kg^{-1})-	
0	27	29
1546	29	28
CONTRAST		
0 vs. 1546 lime	*	NS
C.V.(%)	13	11

* Significant at the 0.05 probability level. NS=Not significant.

Nodule score. Nodule score (Rosas and Bliss, 1984) was affected by the cultivar, lime, Mo, and N source (Table 8-12). In Acacias 4 cultivar, when no lime or Mo were applied, the inoculated treatment showed a higher nodule score than the combined-N or control treatment. This implies that the Rhizobium infected the plants and nodules were formed. Combined N depressed nodulation. This agrees with the findings of Evans (1982) in soybeans, who found that increasing amounts of N to 200 mg kg^{-1} depressed nodulation. Treatments with Mo or lime alone did not affect nodule score for the three N treatments. However, the combination of lime and Mo resulted in similar scores for the inoculated and uninoculated treatments. These two treatments resulted in higher nodule score than the combined N. In Porrillo S. cultivar, no difference in nodule score was found among the three N treatments (inoculated, 0 and 100 N) for the combination of lime and Mo except in the treatment containing Mo without lime where the inoculated treatment was similar to the control in nodule score and higher than the combined-N treatment.

Nodule score was also affected by cultivar, lime, and P (Table 8-13). In Acacias 4 cultivar, similar nodule score was obtained for both P treatments (0 and 80 kg ha^{-1}) with or without the application of lime. For Porrillo S. cultivar, the application of P resulted in higher nodule

score when lime was also applied. Positive P by lime interaction has been reported by Haynes, (1982); Kamprath and Foy, (1985); Sumner and Farina, (1986); Adams, (1980); de Mooy and Pesek, 1966. When no lime was applied, no response to P was measured relative to nodule score.

Bean yield. Bean yield was influenced by the interaction of cultivar, lime, Mo, P, and N source. For the Acacias 4 cultivar (Table 8-14), the inoculated treatment and the control treatment produced similar yields when no lime, P, or Mo was applied. However, the application of N resulted in higher yield (more than 50%) than that from the control or the inoculated treatment. This agrees with findings of Mangual-Crespo et al. (1987), and Fernandes et al. (1983) in field experiments, where beans responded to combined N. When no lime or P were applied with Mo, the inoculated treatment resulted in higher yield than when no Mo was applied. The application of P without lime and Mo resulted in similar yield for the three N treatments. When Mo and P were applied without lime, the combined N treatment resulted in higher yield than the inoculated and the control (50% more). It appeared that Mo had a negative effect on yield without lime. Plants fertilized with combined N, P, and Mo were able to overcome this negative effect.

Table 8-12. Effect of cultivar, lime, Mo, and N source on nodule score (Rosas and Bliss, 1984) for the field experiment from Colindres site in Honduras, 1984.

N applied	Lime ($kg\ ha^{-1}$) 0		Lime ($kg\ ha^{-1}$) 1546	
	Mo ($kg\ ha^{-1}$) 0	Mo ($kg\ ha^{-1}$) 0.5	Mo ($kg\ ha^{-1}$) 0	Mo ($kg\ ha^{-1}$) 0.5
$kg\ ha^{-1}$	Nodule score †			
	Cultivar: Acacias 4			
0	3	3	3	4
100	3	3	3	3
None+inoc.	4	3	3	4
CONTRASTS				
0 N vs. Inoc.	*	NS	NS	NS
100 N vs. Inoc.	NS	NS	NS	*
C.V.(%)	27	39	31	20
	Cultivar: Porrillo S.			
0	3	4	3	3
100	3	2	3	3
None+inoc.	3	3	3	3
CONTRASTS				
0 N vs. Inoc.	NS	NS	NS	NS
100 N vs. Inoc.	NS	**	NS	NS
C.V.(%)	25	39	38	34

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

† Nodule score: 1=poor; 2=below average; 3=average; 4=higher than average; 5=superior.

Table 8-13. EFfect of cultivar, lime, and P on nodulation for the field experiment from Colindres site in Honduras, in 1984.

P applied	Cultivar			
	Acacias 4		Porrillo S.	
	Lime (kg ha^{-1})		Lime (kg ha^{-1})	
	0	1546	0	1546
kg ha^{-1}	-------------Nodule score ┼------------			
0	3	3	3	3
80 P	3	3	3	4
CONTRAST				
0 P vs. 80 P	NS	NS	NS	*
C.V. (%)	33	26	35	33

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

┼ Nodule score: 1=poor; 2=below average; 3=average; 4=higher than average; 5=superior.

The application of lime increased bean yield. Acacias 4 cultivar with the inoculated treatment resulted in higher yield than the control treatment, but lower than the combined N when no P or Mo were applied. The application of Mo tended to reduce yield in the inoculated treatment without P, and once again the treatment with combined N produced higher yield. It appeared that Mo negatively influenced inoculated plants when lime was applied. This could be caused by an excess of Mo availability for the plants caused by liming. This was observed in the limed soil from Colindres where Porrillo S. plant dry weight was depressed by Mo application.

When P was applied with lime but without Mo, the inoculated treatment produced similar results to that of the control and less than 50% of that produced by the combined-N treatment. The addition of Mo, lime, and P increased yield in the inoculated and the control plants. Ruschel et al. (1970) found a similar synergistic relationship among lime, P, and Mo in increasing bean production in Brazil.

When no lime, P, or Mo was applied to Porrillo S. cultivar (Table 8-15) there was no difference among the N treatments. The addition of Mo did not produce differences in yield. When P was added but no Mo or lime, the inoculated treatment produced a higher yield than the control and yield was equivalent to the combined-N treatment. However, the addition of Mo to this treatment caused a decrease in yield.

When lime was applied without P to the Porrillo S. cultivar, inoculated beans produced yields similar to the control. Beans fertilized with combined N resulted in higher yield.

The application of Mo did not have an effect in this case. When P was added along with the lime but without Mo, inoculation resulted in higher yield relative to the control and was comparable to the combined-N treatment. This synergistic effect of lime and P has been mentioned in the literature (Wang and Yuan, 1989; Haynes, 1982; Haynes and Ludecke, 1981; Awan, 1964; Bouton et al., 1981; Sumner, 1979). However, results obtained in the P experiment in 1983 (Chapter 6) did not show this synergism of lime with P in bean plants as reported by Haynes (1982).

The application of lime, P, and Mo resulted in similar responses in yield for the three N treatments. However, with the Porrillo S. cultivar the application of Mo and P increased yield in every N treatment. A positive interaction of Mo, and P has been described by Sumner and Farina, 1986; Adams, 1980; Basak et al., 1982; Doberenier, 1978.

Table 8-14. Effect of the interaction of lime, P, Mo, and N sources on bean yield from Colindres site in Honduras, 1984.

N applied	P ($kg\ ha^{-1}$)			
	0		80	
	Mo ($kg\ ha^{-1}$)		Mo ($kg\ ha^{-1}$)	
	0	0.5	0	0.5
$kg\ ha^{-1}$	--------Bean yield ($kg\ ha^{-1}$)--------			
	Cultivar: Acacias 4 without lime			
0	781	955	1003	643
100	1618	1176	1235	1204
none+inoc.	581	984	1061	498
CONTRASTS				
0 N vs. Inoc.	NS	NS	NS	NS
100 N vs. Inoc.	**	NS	NS	**
C.V. (%)	19	22	25	21
	Cultivar:Acacias 4 with lime			
0	757	893	975	1109
100	1447	1183	1776	1273
none+inoc.	1094	570	871	1006
CONTRASTS				
0 N vs. Inoc.	**	**	NS	NS
100 N vs. Inoc.	**	**	**	NS
C.V. (%)	11	18	19	20

** Significant at the 0.01 probability level. NS=Not significant.

Table 8-15. Effect of the interaction of cultivar, P, lime, Mo, and N source on bean yield from Colindres site in Honduras, 1984.

N applied	P (kg ha^{-1})			
	0		80	
	Mo(kg ha^{-1})		Mo (kg h a^{-1})	
	0	0.5	0	0.5
kg ha^{-1}	--------Bean yield (kg ha^{-1})-------			
	Cultivar: Porrillo S. without lime			
0	620	611	815	887
100	827	896	1421	918
none+inoc.	645	736	1211	832
CONTRASTS				
0 N vs. Inoc.	NS	NS	*	NS
100 N vs. Inoc.	NS	NS	NS	NS
C.V. (%)	22	21	18	23
	Cultivar: Porrillo S. with lime			
0	870	646	701	1145
100	935	893	1155	1447
none+inoc.	738	604	1087	1351
CONTRASTS				
0 N vs. Inoc.	NS	NS	*	NS
100 N vs. Inoc.	**	*	NS	NS
C.V.(%)	16	23	25	18

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

Horticultura Soil

Bean dry weight and yields were lower in this soil than in Colindres soil. Similar results have been obtained in some of the other experiments.

Plant dry weight. Plant dry weight was influenced by cultivar, lime, Mo, P, and N source. For Acacias 4 cultivar (Table 8-17), the minus lime, minus P, and minus Mo treatment induced lower plant dry weight for the inoculated treatment than the control. The combined N-treatment resulted in higher plant dry weight. When Mo was applied but without lime and P, the inoculated treatment produced a higher plant dry weight than the control. Molybdenum application favored plant dry weight of inoculated plants, but was inferior to plant dry weight from the combined-N treatment. This was observed in the pot experiment with Mo in 1984, where the application of Mo in the limed soil from Horticultura resulted in a linear increase in dry weight of the Porrillo S. cultivar (Chapter 7). The combined-N treatment resulted in a higher plant dry weight than the other two N treatments when only Mo was applied. This suggested a positive interaction of Mo with combined N. When P was applied but no lime or Mo, the three N treatments produced a higher yield than when no P was applied. The application of P and Mo without lime resulted in similar

Table 8-16. Analysis of variance for Horticultura site experiment in Honduras, 1984.

Source of variation	df	Dry wt	Grain yield	Total N	Nod.
		---------Probability ---------			
Replicate	1	NS	NS	NS	NS
Cultivar (C)	1	**	NS	NS	NS
Lime (L)	1	**	**	**	**
Phosphorus (P)	1	**	NS	NS	**
L*P	1	NS	*	NS	NS
Molybdenum (Mo)	1	**	NS	NS	NS
L*Mo	1	**	NS	NS	NS
Mo*P	1	**	NS	NS	NS
L*Mo*P	1	**	NS	NS	NS
N	2	**	**	NS	NS
L*N	2	NS	**	NS	NS
P*N	2	**	NS	NS	NS
L*P*N	2	NS	NS	NS	*
Mo*N	2	**	NS	NS	NS
L*Mo*N	2	NS	NS	NS	NS
Mo*P*N	2	**	**	NS	NS
L*Mo*P*N	2	NS	**	NS	NS
C*L	1	NS	NS	**	NS
C*P	1	NS	**	NS	NS
C*Mo	1	NS	**	NS	*
C*N	1	**	NS	NS	NS
C*L*P	1	NS	NS	NS	NS
C*L*Mo	1	NS	NS	NS	NS
C*L*N	2	**	NS	NS	NS
C*Mo*P	1	*	NS	NS	NS
C*P*N	2	NS	NS	**	NS
C*Mo*N	2	NS	**	*	*
C*L*Mo*P	1	**	NS	NS	NS
C*L*P*N	2	*	*	NS	NS
C*Mo*P*N	2	**	NS	NS	NS
C*L*Mo*N	2	NS	NS	NS	NS
C*L*Mo*P*N	2	**	NS	NS	NS

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS= Not significant.

Table 8-17. Influence of the interaction of cultivar, lime, Mo, P, and N source on plant dry weight from Horticultura site in Honduras, 1984.

N applied	P ($kg\ ha^{-1}$)			
	0		80	
	Mo ($kg\ ha^{-1}$)		Mo ($kg\ ha^{-1}$)	
	0	0.5	0	0.5
$kg\ ha^{-1}$	----Bean plant dry weight (g/5 plts)----			
	Cultivar: Acacias 4 without lime			
0	13	6	18	17
100	17	23	26	39
none+inoc.	10	10	19	16
CONTRASTS				
0 N vs. Inoc.	**	**	NS	NS
100 N vs. Inoc.	**	**	NS	**
C.V.(%)	4	7	34	16
	Cultivar:Acacias 4 with lime			
0	10	7	9	24
100	12	40	43	35
none+inoc.	13	16	13	22
CONTRASTS				
0 N vs. Inoc.	**	**	**	NS
100 N vs. Inoc.	NS	**	**	*
C.V.(%)	7	14	7	15

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

plant dry weight for the inoculated and the control, but the combination of P, Mo, and combined N without lime resulted in higher plant dry weight than the other two N treatments. When lime was applied to Acacias 4 cultivar, but without P or Mo, the inoculated treatment induced higher plant dry weight than the control. Dry weight obtained was equal to that obtained from the combined-N treatment. Similarly, when Mo was applied with lime but without P, the inoculated treatment enhanced plant dry weight more than the control treatment. However, the combination of lime, Mo, and combined N without P resulted in more than double the plant dry weight from the inoculated treatment.

The application of P, and lime without Mo resulted in higher plant dry weight for the inoculated treatment when compared to the control. However, the combined N resulted in more than three times higher plant dry weight than the other two N treatments. When the Acacias 4 cultivar was supplied with lime, P, and Mo, plant dry weight from the inoculated treatment increased but was similar to that from the control treatment. Combined N resulted in higher plant dry weight. In this case, combined N produced lower plant dry weight than when Mo was omitted. Similar results were reported for bean yield in Table 8-14 for the Acacias 4 cultivar with lime in Colindres soil.

For the Porrillo S. cultivar, the minus lime, minus Mo, and minus P treatment produced plant dry weight similar for the three N treatments (Table 8-18). The application of Mo tended to decrease plant dry weight in the control and inoculated treatments. However, this did not happen when combined N was applied. A synergistic relationship between combined N and Mo was found by Parker and Harris (1977) in soybeans. A similar situation was shown when plants were fertilized with P but not lime or Mo. The treatment with combined N always produced higher plant dry weight. When Mo and P were applied without lime, the inoculated treatment produced even lower plant dry weight than the control. It appeared that Mo had a negative effect on inoculated plants when no lime was applied.

The application of lime for Porrillo S. cultivar without P or Mo produced similar dry weight for the three N treatments. Weights were higher than when lime was omitted. Lime with Mo but without P resulted in similar plant dry weights for the control and inoculated treatments. The combined-N treatment produced higher plant dry weight but less than the same treatment without lime. The addition of P and lime without Mo resulted in an increase in plant dry weight in the inoculated treatment relative to the control

Table 8-18. Effect of lime, P, Mo, and N source on Porrillo Sintetico plant dry weight from the Horticultura site in Honduras, 1984.

N applied	P ($kg\ ha^{-1}$)			
	0		80	
	Mo ($kg\ ha^{-1}$)		Mo ($kg\ ha^{-1}$)	
	0	0.5	0	0.5
$kg\ ha^{-1}$	----Bean plant dry weight (g/5 pls)-----			
	Cultivar: Porrillo Sintetico without lime			
0	10	7	16	19
100	11	26	28	24
none+inoc.	11	7	14	11
CONTRASTS				
0 N vs. Inoc.	NS	NS	NS	*
100 N vs. Inoc.	NS	**	**	**
C.V.(%)	27	10	11	16
	Cultivar: Porrillo Sintetico with lime			
0	14	11	7	20
100	18	17	13	37
none+inoc.	16	9	17	22
CONTRASTS				
0 N vs. Inoc.	NS	NS	**	NS
100 N vs. Inoc.	NS	**	NS	*
C.V.(%)	15	23	23	22

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

and the combined N treatments. The application of Mo increased plant dry weight when this element was applied with lime, P, and N; however, with inoculation, results similar to the control were obtained. This positive effect of lime and Mo on Porrillo S. plant dry weight was also observed for the Mo pot experiment in 1984 with the limed Horticultura soil.

Total N content. Plant tissue N content was influenced by cultivar, lime, Mo, and P (Table 8-19). Plant tissue N content was similar for all the combinations of lime, Mo, P, and cultivar except when no lime was applied to soil used to grow Porrillo S. plants, the N content was lower when P was applied without Mo. When P and Mo were applied, plant tissue N content increased. This synergistic relation of P and Mo has been reported by Doberenier (1978).

Total N content was also affected by the interaction of cultivar, P, and N source (Table 8-20). The total N content was similar in all combinations of cultivar, P, and N source except for Porrillo S. cultivar were plants from the combined-N treatment with P applied contained a higher N content than those from the other two N treatments. Phosphorus has been reported to act synergistically with N to produce higher total N in plants (Jimenez and Villalobos, 1980).

Table 8-19. Effect of cultivar, lime, Mo, and P on bean total plant N content on the Horticultura site in Honduras, 1984.

P applied	Lime (kg ha^{-1})			
	0		1593	
	Mo (kg ha^{-1})		Mo (kg ha^{-1})	
	0	0.5	0	0.5
kg ha^{-1}	Total plant N (g kg^{-1})			
	Cultivar: Acacias 4			
0	28	31	31	30
80	28	30	30	30
CONTRAST				
0 P vs. 80 P	NS	NS	NS	NS
C.V.(%)	15	12	8	12
	Cultivar: Porrillo Sintetico			
0	30	26	32	31
80	26	29	31	31
CONTRAST				
0 P vs. 80 P	**	**	NS	NS
C.V.(%)	8	8	6	9

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

Nodule score. Nodule score was affected by cultivar, Mo, and N source (Table 8-21). Nodule score was similar for all combinations of cultivar, Mo, and N treatments except for the Porrillo S. cultivar with Mo where nodule score from the inoculated treatment was higher than with the combined-N treatment. This is in agreement with that observed in the 1982 field experiment (Table 8-3). Inhibition of nodulation by combined N is common (Evans, 1982; Graham, 1981).

Nodule score was also affected by the combination of lime, P, and N source (Table 8-22). Where no lime or P was applied a lower nodule score for the combined-N treatment was obtained when compared to the inoculated treatment. When P was applied without lime, the inoculated treatment resulted in a nodule score similar to that from the combined-N treatment and lower than the control. Although the application of P and lime resulted in a higher nodule score than without applied P, no differences among the N treatments were observed with and without P.

Bean yield. Bean yield was lower than in the Colindres soil. However, data were more consistent with respect to the superiority of combined N over inoculated and control treatment than in the Colindres soil. Yield was affected by cultivar, lime, P, and N source (Table 8-23). For the Acacias 4 cultivar, when no lime and no P were applied, the inoculated treatment produced a yield similar to the control treatment. Combined N produced the highest yield of the

three N treatments. When P was applied without lime, the inoculated treatment produced a higher yield than the control. However, it was inferior to the combined-N treatment. The application of lime with or without P produced a similar response for the control and inoculated treatment. The response to the combined-N treatment was enhanced by the application of P and lime.

For the Porrillo S. cultivar, when no lime was applied, P did not influence bean yield relative to the control and the inoculated treatment. The combined-N treatment produced a higher yield than the other two N treatments. When lime was applied without P no differences among the N treatments were observed. Similar results were noted when P was applied. Treatment with combined N resulted in higher yields with and without P.

Table 8-20. Effect of cultivar, P, and N source on total N content on the Horticultura site in Honduras, 1984.

N applied	Cultivar			
	Acacias 4		Porrillo S.	
	P (kg ha^{-1})		P (kg ha^{-1})	
	0	80	0	80
kg ha^{-1}	----Total plant N content (g kg^{-1})----			
0	31	28	30	29
100	29	30	30	31
none+inoc.	31	29	29	28
CONTRAST				
0 N vs. Inoc.	NS	NS	NS	NS
100 N vs. Inoc.	NS	NS	NS	**
C.V. (%)	11	12	10	9

** Significant at the 0.01 probability level. NS=Not significant.

Table 8-21. Effect of cultivar, Mo, and N source on bean nodule score on the Horticultura site in Honduras, 1984.

N applied	Cultivar			
	Acacias 4		Porrillo S.	
	Mo (kg ha^{-1})		Mo (kg ha^{-1})	
	0	0.5	0	0.5
kg ha^{-1}	---------Nodule score † ------------			
0	3	4	3	4
100	3	4	3	2
none+inoc.	3	4	3	4
CONTRASTS				
0 N vs. Inoc.	NS	NS	NS	NS
100 N vs. Inoc.	NS	NS	NS	**
C.V.(%)	22	28	28	33

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=Not significant.

† Nodule score: 1=poor; 2=below average; 3=average; 4=higher than average; 5=superior.

Table 8-22. Effect of lime, P, and N source on bean nodule score on the Horticultura site in Honduras, 1984.

N applied	Lime (kg ha^{-1})			
	0		1593	
	P (kg ha^{-1})		P (kg ha^{-1})	
	0	80	0	80
kg ha^{-1}	----------Nodule score ┼	--------------		
0	3	4	3	4
100	2	3	3	4
none+inoc.	3	3	3	4
CONTRASTS				
0 N vs. Inoc.	NS	*	NS	NS
100 N vs. Inoc.	*	NS	NS	NS
C.V.(%)	26	24	23	18

* Significant at the 0.05 probability level. NS=Not significant.
┼ Nodule score: 1=poor; 2=below average; 3=average; 4=higher than average; 5=superior.

Table 8-23. Effect of cultivar, lime, P, and N source on bean yield on the Horticultura site in Honduras, 1984.

N applied	Lime ($kg\ ha^{-1}$)			
	0		1593	
	P ($kg\ ha^{-1}$)		P ($kg\ ha^{-1}$)	
	0	80	0	80
$kg\ ha^{-1}$	Bean yield ($kg\ ha^{-1}$)			
	Cultivar:Acacias 4			
0	230	234	268	307
100	647	627	556	685
none+inoc.	233	352	331	400
CONTRASTS				
0 N vs. Inoc.	NS	*	NS	NS
100 N vs. Inoc.	**	**	**	**
C.V.(%)	18	22	29	19
	Cultivar:Porrillo S.			
0	289	262	366	376
100	697	533	482	594
none+inoc.	226	199	426	345
CONTRASTS				
0 N vs. Inoc.	NS	NS	NS	NS
100 N vs. Inoc.	**	**	NS	**
C.V.(%)	31	24	29	23

** Significant at the 0.01 probability level. NS=Not significant.

Bean yield was also influenced by the interaction of cultivar, Mo, and N source (Table 8-24). There were no differences between the inoculated treatment and the control for the two cultivars with or without the application of Mo. Bean yield was not influenced by Mo. For both cultivars, the combined-N treatment was superior to the other two N treatments.

Bean yield was affected by lime, P, Mo, and N source (Table 8-25). When lime was omitted, the inoculated treatment produced results similar to the control treatment with and without P or Mo. In all combinations of P and Mo, the combined-N treatment resulted in higher yields than the control and the inoculated treatment. When lime but no P or Mo was applied, the three N treatments resulted in similar yields. However, in all other combinations of lime, P, and Mo, the inoculated treatment and control produced similar yields. They were lower than the yield obtained with combined N. The synergistic relationship among lime, P, and Mo on bean yield has been reported previously by Ruschel et al. (1970).

Table 8-24. Effect of cultivar, Mo, and N source on bean yield on the Horticultura site in Honduras, 1984.

N applied	Cultivar			
	Acacias 4		Porrillo S.	
	Mo (kg ha^{-1})		Mo (kg ha^{-1})	
	0	0.5	0	0.5
kg ha^{-1}	--------Bean yield (kg ha^{-1})--------			
0	314	296	307	340
100	674	584	519	633
none+inoc.	295	273	316	282
CONTRASTS				
0 N vs. Inoc.	NS	NS	NS	NS
100 N vs. Inoc.	**	**	**	**
C.V.(%)	26	23	38	24

** Significant at the 0.01 probability level. NS= Not significant.

Table 8-25. Effect of lime, P, Mo, and N source on bean yield on the Horticultura site in Honduras, 1984.

N applied	P ($kg\ ha^{-1}$)			
	0		80	
	Mo ($kg\ ha^{-1}$)		Mo ($kg\ ha^{-1}$)	
	0	0.5	0	0.5
$kg\ ha^{-1}$	--------Bean yield ($kg\ ha^{-1}$)-------			
	Without lime			
0	214	305	306	307
100	648	696	602	557
none+inoc.	264	195	176	256
CONTRASTS				
0 N vs. Inoc.	NS	NS	NS	NS
100 N vs. Inoc.	**	**	**	**
C.V.(%)	25	25	32	13
	With lime			
0	438	259	284	399
100	455	582	681	598
none+inoc.	407	288	374	370
CONTRASTS				
0 N vs. Inoc.	NS	NS	NS	NS
100 N vs. Inoc.	NS	**	**	**
C.V.(%)	27	24	24	16

** Significant at the 0.01 probability level. NS= Not significant.

Conclusions

1982

On the Horticultura site, inoculation produced better nodulation than either the absolute control treatment (without any nutrient) or the complete treatment (with supplemental lime, P, Mo, and N). However, plant dry weight was superior when combined N, P, lime, and Mo were applied. No other parameters were affected.

1983

On the Colindres site, plant dry weight, height, and total plant N were increased by the application of P. Cultivars responded differently to P; Porrillo S. plants were taller than Acacias 4 with applied P. There was also a synergistic relation between P and combined N that produced more total N in the plant tissue. In most of the cases, combined-N treatments were superior to the inoculated treatments; this was especially observed in bean yield. Molybdenum tended to act positively on inoculated plants, but decreased plant dry weight in the control and combined N treatments.

1984

In general, combined-N treatments resulted in higher yield than inoculated in both sites. This was more consistent on the Horticultura than on the Colindres site. On the Colindres site, responses to treatments were higher

than in the Horticultura site, especially with respect to plant dry weight and grain yield, indicating a better environment for bean growth.

Although plants for the three N treatments were nodulated, inoculated treatments almost always resulted in lower yields. According to Vincent (1970) and Date (1982), if plants from the control and the inoculated treatments are nodulated but with poor growth relative to N-fertilized plants this is an indication that either the inoculum strain is "out-competed" by native strains or the inoculum strains are ineffective. More research is needed to determine which of these situations was present in Honduran soils.

On the Colindres site, both cultivars, Acacias 4 and Porrillo S., showed similar plant dry weight and total N content. The Porrillo S. cultivar produced a higher response to the application of lime and P than the Acacias 4 cultivar. The Acacias 4 cultivar produced a higher yield as a response to combined N. Lime did not influence plant dry weight. However, lime increased total plant N content, nodule score, and yield of both cultivars. Applied P increased plant dry weight and yield but did not influence total N content in the plant, or nodule score. Yield of inoculated plants was increased when P was applied without lime. Molybdenum applications produced mixed results; a negative effect was observed in bean plant dry weight when Mo was applied with lime, especially in the inoculated

treatments. In the combined-N treatments, Mo did not have a consistent effect. Molybdenum interaction with P was positive for Acacias 4 plant dry weight for the inoculated plants, but no response was obtained with combined N. It has been suggested that Mo applications can correct N deficiency and provide Mo for nitrate reduction but not for N_2 fixation. The Porrillo S. cultivar appeared to be more enhanced by Mo than the Acacias 4 cultivar. The triple interaction of lime, P, and Mo produced increased yields from the inoculated and combined-N treatments.

On Horticultura site, cultivars were similar in plant dry weight, total N content, nodule score, and yield. Lime applications increased plant dry weight, nodule score, and yield of inoculated plants. Lime did not affect the yield of plants fertilized with combined N. The application of P increased plant dry weight, and nodule score. No effect of P on total N content was obtained. Yield increased when P was applied in both the inoculated and combined-N treatments for Acacias. For the Porrillo S. cultivar this response was not observed. Application of Mo increased plant dry weight of Acacias 4 plants treated with combined N. Response to Mo was higher when lime was applied. No response to Mo was observed when P was applied. Yield was not influenced by applications of P. A positive effect of P on bean plant dry weight, but not yield has also been reported by Chaib et al. (1984).

In conclusion, none of the corrective measures taken (Mo, lime, or P) made inoculation a viable alternative in yield to combined N. No economical analysis was made, but logistical problems with significant costs, make it unlikely that the technology resulting in lower yields would be attractive to farmers.

CHAPTER 9
EFFECT OF IRRIGATION ON BIOLOGICAL NITROGEN FIXATION AND BEAN YIELD

Introduction

Water is an essential factor for all plants. However plants differ in their water requirement and in their tolerance to water stress (Sponchiado et al., 1989). Sinclair et al. (1988) found that mild drought treatments imposed on soybeans did not affect nodule number or dry weight. Differences were observed only when severe drought was imposed compared to the well-watered and mildly drought-stressed treatments. On the other hand, bean plants are very sensitive to soil-water conditions and yield can suffer greatly from even brief periods of a water shortage.

Very few studies concerning the effect of water stress on BNF were found in the literature; most of them were related to bean growth and yield. Beans seem to suffer from water stress at all growth stages (Halterlein, 1983; D'Souza and Coulson, 1988), however, stress from just before flowering through pod fill apparently causes the most severe

damage. The plants can, at least partly, overcome the effects of water deficits during the vegetative stages of growth. Bean flowering, when N_2 fixation is at near maximal, is also a critical period of probable water stress in almost 60% of the beans produced in Latin America (CIAT, 1979). Sub-optimum amounts of water may reduce photosynthesis, restrict the transport of fixed N_2 from the nodule and reduce fixation by inhibition of oxygen diffusion through the nodule cortex (Roughley, 1972).

The determination of water effect on bean growth and its association with Rhizobium and the corresponding N_2 fixation under Honduran conditions was important to include in this research, since water is one of the most limiting factors in any crop production in the tropics, especially those crops that depend on rainfall. The objective of this experiment was to determine the effect of different irrigation levels and two planting dates on BNF and yield of beans. This field experiment was conducted during the dry season in order to manipulate moisture conditions via different irrigation regimes.

Materials and Methods

The experiment was conducted at Agronomia, at the Escuela Agricola Panamericana in Honduras. Soil characteristics are presented in Table 3-2. A general

fertilizer application was made using 80 kg P ha^{-1} from triple superphosphate (46% P_2O_5) and 0.5 kg Mo ha^{-1} from sodium molybdate (39% Mo). No combined N was applied.

Plots of 4 by 2 m consisting of 8 rows each were planted on 23 Feb. and 9 Mar. 1983 to determine the effect of different water regimes and planting dates on bean growth and yield. Inoculant mixture (equal parts of exotic and indigenous Rhizobium strains) was placed next to the Acacias 4 cultivar seed at the rate of 9.2 kg ha^{-1}. Rhizobia population was determined to be 6.54 x 10^8 rhizobia per g of inoculant. The seeds and inoculant were covered with about 3 cm of soil.

The five treatments consisted of differential irrigation as follows:

1) Application of low irrigation level (100 L $plot^{-1}$) estimated to be roughly 1/3 capacity during the first 48 d and then plants were irrigated with 300 L $plot^{-1}$ that was estimated to approximate full field capacity.

2) Application of medium irrigation level (200 L $plot^{-1}$) estimated to approximate to be 2/3 field capacity during the first 48 d, after that plants were irrigated with 300 L $plot^{-1}$ that was estimated to be approximate full field capacity.

3) Application of low irrigation level (100 L $plot^{-1}$) to approximate 1/3 field capacity during the entire plant cycle.

4) Application of medium irrigation level (200 L $plot^{-1}$) to simulate 2/3 field capacity during the entire plant cycle.
5) Application of high irrigation level (300 L $plot^{-1}$) to approximate full field capacity was applied during the entire plant cycle.

Calculations of required water for this experiment determined that to obtain the amount of water needed one should irrigate 5, 10, and 15 min (100, 200, and 300 L water $plot^{-1}$) every 2 d to approximate one third, two thirds and full field capacity, respectively (Hansen et al., 1980). Soil moisture was assumed to be nil (See Appendix C for rainfall distribution). The amount of water to be applied was determined using the following formula:

QT=10 Ad

Where Q= caudal (L/min)

T= period of time (min)

A= area (m^2)

d= water retention capacity

Q = 11 m per gallon= 19 L per 1 min.

For a sandy loam soil and root depth of 0.3 m d was calculated as following:

d= (12 cm/m) x 0.3 m = 3.6 cm of water that soil retained for that depth.

Allowable depletion (1/3 field capacity)=33%

d=3.6 x 0.33= 1.19 cm water lamina

A= 8 m^2

$T=(10 \times 8 \times 1.19)/19= 5$ min

5 minutes = 1/3 field capacity (low level)

10 minutes = 2/3 field capacity (medium level)

15 minutes = 3/3 field capacity (high level)

The frequency of irrigation was determined in the following manner:

$ET_c=Kc \times ETp$

where ET_c= Crop evapotranspiration

$Kc=0.9$ (Crop coefficient)

ETp= Potential evapotranspiration (177 mm for March)

$ET_c=0.9 \times 177= 159.3$ mm per month

$ET_c=5.3$ mm per d

irrigation frequency= d/ET= 1.19/5.3= 2.2 d

Five plants in each of the border rows were collected at flowering (at 48 d) stage to determine dry weight of plants and nodules, and N in plant tissue. Grain yield (at 14% moisture) at harvest was recorded.

Treatments were arranged in a split-plot design with two planting dates as main plots and irrigation intensity as subplots. A completely randomized block design was used with four replicates for each planting date. Statistical analyses were conducted using the Statistical Analysis System (SAS, 1987).

Results and Discussion

Foliar samples from this experiment were disposed by an involuntary error, thus, N in tissue could not be determined to study the effect of BNF. However, nodule dry weight, plant dry weight and yields were recorded. An analysis of variance for this experiment is shown in Table 9-1. Data are presented in Table 9-2. Main effects of irrigation treatments were observed on all parameters studied; planting date affected bean yield. The second planting date produced higher bean yield than the first planting date (1,350 versus 940 kg ha^{-1}, respectively) but did not influence bean plant or nodule dry weight. Effect of planting date on bean yield could be related to an environmental factor not included in this study, such as day length and temperature. Compared to other experiments, flowering was delayed for approximately 7 d, probably due to a combination of photoperiod sensitivity of bean plants and to the water treatments as has been reported by Graham (1981) and Halterlein (1983). Bean sensitivity to photoperiod had been used to delay flowering, which could have enhanced nodulation, and hence, more accumulation of N that could result in higher yields.

Plant dry weight. Plant dry weight at flowering stage (R6) was greatly influenced by the imposed water regime

intensities (Table 9-2). The treatments which included the application of the low irrigation level applied during the first 48 d or during the entire cycle resulted in the lowest plant dry weight. Lack of water during the first 48-d period was critical for plant growth because it coincided with flowering and initiation of pod formation (Halterlein, 1983). The medium level of irrigation in which water was applied to maintain the soil approximately to 2/3 field capacity during the entire crop cycle produced no differences in plant dry weight relative to the high water level treatment during the entire cycle. In irrigated soybeans, Smith and del Rio Escurra (1982) found higher plant dry weight as well as nodule dry weight in relation to non-irrigated plants. They reported that soil temperature at 2.5 cm deep in the dry plots was 3 to 8 ^{0}C hotter than the maximum temperature in the irrigated plots. A similar situation could have taken place in this experiment, since it was conducted during the hottest period of the year in Zamorano.

Nodule dry weight. This parameter was affected by water treatments (Table 9-2). Saito et al. (1979) reported nodule weight 5 to 10 times higher in wet soils than in dry soils. A decrease in nodule numbers has been determined in beans to be due to water stress during the vegetative period (Quintero et al., 1983). In this experiment, the application of low (5 min) or medium (10 min) level of water to maintain

at 1/3 or 2/3 field capacity during the first 48 d, respectively, followed by application of high level of water to approximate full field capacity or the application of low level of water for the entire cycle did not produce differences in the nodule dry weight when compared to the high water treatment. However, when water was applied to obtain 2/3 of field capacity during the entire cycle, higher nodule dry weight was obtained than in the full field capacity treatment. This supports the opinion of several authors (Sprent, 1976; Roughley, 1972; Freire, 1984), that excess of water decreases nodulation because the plant roots lack adequate oxygen. Also, nodules are more prone to rot in the presence of excess water. This was observed in treatments where larger amounts of water were applied.

Yield. Bean yield was also affected by water treatments. The treatment which included water to maintain 2/3 field (medium) capacity during the first 48 d and then maintained to full field capacity was not different from the treatment which included application of equal quantities of water continuously during the entire cycle. These two treatments also produced bean yield similar to the treatment which included high level of water to maintain the soil at full field capacity (Table 9-2). These data imply that once that amount of water needed to maintain the soil at 2/3 field capacity is applied the results are equivalent to the treatment where water is applied to maintain the soil at

full field capacity. Bierhuizen and de Vos (1959) cited by Halterlein (1983) found that if water stress happened during the vegetative period plants were stunted, but did not decrease yield if adequate moisture was maintained during flowering. Kriegbaum (1967) also cited by Halterlein (1983) determined that water applied during the period that comprised from germination until before flowering had no effect on yield.

Nodulation is not always correlated with higher yield. In this study no correlation was found between nodulation and yield. Plant dry weight was correlated to yield and to nodulation. Similar results in beans has been reported also by Edje et al. (1975).

Table 9-1. Analysis of variance for the moisture experiment in Honduras, 1983.

Source	df	Plant dry wt MS	Plant dry wt F	Plant nod wt MS	Plant nod wt F	Grain yield MS	Grain yield F
Planting date	1	193.16	NS	18922	NS	1717688	*
Rep (Planting)	6	173.48		26715		259536	
Water treatments	4	374.89	**	21444	NS	1155719	**
Planting x water	4	19.20	NS	15255	NS	193755	NS
Error	24	32.65		14050		136103	
Total	39						
C.V.(%)		40		42		32	

** Significant at the 0.01 probability level. NS=Not significant.

Table 9-2. Moisture effect on bean plant and nodule dry weight of five plants at 48 d, and yield at harvest for the moisture experiment in Honduras, 1983.

Treatments	Plant dry wt	Plant nod. dry wt	Grain yield
	(g/5 plts)	(mg/5 plts)	(kg ha^{-1})
1) Low for 48d+high	6.35	280	809
2) Medium for 48d +high	11.75	296	1252
3) Low entire cycle	10.56	279	679
4) Medium entire cycle	19.01	331	1463
5) High entire cycle	23.64	230	1513
CONTRASTS			
Treat 1 vs. 5	**	NS	**
Treat 2 vs. 5	**	NS	NS
Treat 3 vs. 5	**	NS	**
Treat 4 vs. 5	NS	**	NS
C.V. (%)	40	42	32

** Significant at the 0.01 probability level. NS= Not significant.

Conclusions

Bean plants are very sensitive to water stress; and yield can suffer greatly from even a brief period of water shortage, especially at flowering (Halterlein, 1983). The application of a medium level of water to maintain 2/3 field capacity during the entire cycle resulted in maximum nodulation. Yields obtained when a medium level of water was applied to approximate 2/3 field capacity during the entire cycle were equivalent to those of the full field capacity treatment.

The application of a low level of water, the driest irrigation treatment reduced plant dry weight and yield. Nodule dry weight was affected by the different water treatments; a medium level of water (2/3 field capacity) during the entire cycle resulted in superior nodule dry weight, indicating that it was the optimum for nodulation. This treatment also produced a yield equivalent to the yield produced by the high water treatment. The latter treatment depressed nodulation, and hence, BNF would be decreased with excess of water. Although yield was higher when the highest water level was applied, this was not different from the medium water treatment. In conclusion, bean growth, nodulation and yield were maximized by maintaining the soil

at 2/3 of field capacity (10 minutes). From an economic point of view this is the recommended treatment; it is less expensive than maintaining the soil at full field capacity (15 minutes), because it requires less water, less labor costs are involved, and less risky than full irrigation which could cause a yield reduction as was noted for chickpea plants in India (Parihar and Tripathi (1989). This yield reduction resulted from excessive vegetative growth at the expense of pod formation.

Even though combined N was not applied in this experiment, yields were equivalent to the ones obtained when combined N was applied in previous studies. This strongly suggest that water is a key factor in bean production.

CHAPTER 10
EFFECT OF TIMING OF APPLICATION OF AMMONIUM SULFATE ON MAIZE (*Zea mays* L.) GROWTH AND YIELD

Introduction

Maize (*Zea mays* L.) is the most important source of carbohydrates for Central American people. It is also an important animal food. Regional average yield for maize in Central America is 1,400 kg ha^{-1}, 22% of the U.S. yield (Leonard, 1987). A large part of the maize is produced by farmers with limited resources and few advanced technological inputs. One of the most important practices is the use of fertilizer. According to their resources, farmers may apply complete fertilizers (with N and P and sometimes K) to maize in either of two ways. The farmer may prepare the land, then apply the total amount of P along with part of the N at planting time in the bottom of the furrow, lightly cover the fertilizer with soil, drop the maize seeds into the trench and then cover them with soil. A second way involves a sidedress application of one half of the N and all the P 10 to 15 d after plants have emerged. This strategy is used in order to avoid loss of fertilizer in

case the seeds do not germinate or are destroyed by pests. In both cases, the second application of N is made 25 to 45 d after planting at which time the plants are "hilled up".

A common fertilizer used in the region is ammonium sulfate. Some of the advantages of ammonium sulfate include its low hygroscopicity, chemical stability, and agronomic suitability (Tisdale and Nelson 1985). Taking into account that Central American soils are deficient in S, ammonium sulfate is an inexpensive and readily available source of both nutrients. This compound contains 24% S. Its major short-term disadvantage is not the acidic reaction that is formed in the soil, but the salt concentration that is formed in the soil close to the seed; these salts could damage the maize plants, especially seedlings. In Central America, the planting of seeds close to the fertilizer is not recognized as being potentially harmful for the seedlings.

The objective of this experiment was to determine the effect of ammonium sulfate at different times on maize growth and yield.

Materials and Methods

The experiment was conducted at the Escuela Agricola Panamericana situated in the Yeguare Valley, 37 km from Tegucigalpa, Honduras. Some of the chemical characteristics of the soils are presented in Table 3-3.

Maize was planted at three locations --Colindres, Agronomia, and San Nicolas-- in June 1984 using Dekalb B666 hybrid maize seed to obtain 44,000 plants ha^{-1}. Plots measuring 4 by 5 m were fertilized with P (90 kg ha^{-1}) as concentrated superphosphate (46% P_2O_5). Nitrogen was applied as ammonium sulfate (21% N) according to the treatments shown in Table 10-1. In treatment 1, N was split in three parts to minimize the possibility of salt toxicity. The first part was applied at planting, then the second part 15 and 45 d after planting. In treatment 2, the N was split in two parts; the first one (equivalent to 100 kg N ha^{-1}) was applied at planting and the second (20 kg ha^{-1}) was applied 45 d after planting. This treatment was included to determine if salt toxicity occur. Treatment 3 consisted of splitting the N in two parts; the first half was applied 15 d after planting and the second half was applied 45 d after planting. This treatment was a modification of the farmer option and it has been suggested by Jacome and Blue (1980). Treatment 4 consisted of splitting the N in two parts; the first one (50 kg ha^{-1}) was applied at planting, the other part (70 kg ha^{-1}) was applied 30 d after planting. This treatment is the one that Centro Nacional de Tecnologia Agropecuaria (CENTA/MAG) recommended (Salazar et al., 1979). In all treatments, the P was applied at planting. Treatments were replicated six times at each location. Treatments were identified in the following way:

1) N-split in three parts to minimize possibility of seed toxicity
2) Test for salt toxicity. Treatment to determine if salt toxicity occurs.
3) A modification of farmer option suggested by Jacome and Blue (1980), to minimize adverse effects of fertilizer on maize seeds and seedlings.
4) Treatment recommended by CENTA

A split-plot design with locations representing the main plots and treatments the subplots was employed. Plant and root dry weight 15 d after planting, as well as grain yield at 12% moisture were recorded. Statistical analyses were made according to the SAS procedures (SAS, 1987).

Table 10-1. Nitrogen treatments used in the maize experiment at Zamorano, Honduras, 1984.

Treatment †	Days after planting			
	0	15	30	45
	N applied (kg ha^{-1})			
1	50	35	-	35
2	100	-	-	20
3	-	60	-	60
4	50	-	70	-

† Application at planting was in the bottom of a trench, where the fertilizer and seed were placed to the side of the plants, then cover with soil. Other applications were sidedressings where the N fertilizer was placed 20 cm to the side of the plants, then covered by soil.

Results and Discussion

An analysis of variance of this experiment is presented in Table 10-2. Means for plant dry weight, root dry weight, plant height, and grain yield are presented in Table 10-3.

Plant dry weight. Maize plant dry weights 15 d after planting were affected by treatments (Table 10-3). The treatment suggested by Jacome and Blue (1980) resulted in a lower plant dry weight than the other three treatments. No differences were observed between three applications versus two applications. Application of a high rate of ammonium sulfate at 0 d produced higher dry weight than treatment 3.

Root dry weight. Similar to plant dry weight, the Jacome and Blue-suggested treatment produced lower root dry weight (Table 10-3). No apparent salt toxicity was observed when compared to treatment 2 (high amount of ammonium sulfate at planting) was compared with treatments 3 and 4 (with low amounts of this fertilizer at planting); furthermore, root stimulation by NH_4^+ application has been documented by Anghinoni and Barber (1988, 1990). No differences were found between splitting N in three applications versus two applications.

Plant height. Maize plant height was also affected by treatments (Table 10-3). The tallest plants were obtained when treatment 1 and 2 were applied, followed by treatments 4 and 3.

Maize yield. Grain yield was not affected by treatments at the three locations (Table 10-3). According to these data, applying ammonium sulfate at planting had favorable effects on seedling weight, root weight, and height; and the lack of N in the soil depressed the growth of maize seedlings at 15 d. However, plants which received the later N application were able to recuperate and produce a yield similar to those from other treatments.

Table 10-2. Analysis of variance for the maize experiment at the three locations in Honduras, 1984.

Source	df	Yield MS	Yield F	Root dry wt MS	Root dry wt F	Plant dry wt MS	Plant dry wt F	Height MS	Height F
Rep	5	260442	**	0.66	NS	0.96	NS	9.7	NS
Location	2	128767	NS	0.99	NS	1.20	NS	57.3	NS
Loc x rep	10	104899		1.28		0.70		25.6	
Treatments	3	30125	NS	4.14	**	6.10	**	65.4	**
Loc x treat	6	30361	NS	0.17	NS	0.90	NS	9.2	NS
Error	45	47118		0.49		0.65		7.7	

** Significant at the 0.01 probability level. NS=Not siginificant.

Table 10-3. Mean plant and root dry weight, height of five maize plants (15 d post-plant), and yield for the maize experiment in Honduras, 1984.

Treatment	Plant characteristics at 15 d: Top dry wt	Root dry wt	Height	Grain yield
	(g/5 plts)	(g/5 plts)	(cm)	(kg ha^{-1})
1	1.99	1.77	9.1	1380
2	1.96	1.82	8.9	1270
3	0.74	0.78	5.0	1340
4	1.54	1.42	7.1	1320
CONTRASTS				
Treatments				
1 vs. 2 3 4	**	*	**	NS
3 vs. 4	**	**	*	NS
2 vs. 1 3 4	*	**	*	NS

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. NS=not significant.

Conclusions

No yield differences were observed despite the huge differences at 15 d in plant dry weight, root dry weight, and plant height. At 15 d, treatment 3 showed the lowest plant dry weight, root dry weight, and height of plants in Colindres and Agronomia. In San Nicolas, maize plants did not show this; probably this last site had more available N than the other two sites. However, this seedling effect did not influence the yield; the three locations produced similar yields.

No salt toxicity was observed when comparing intermediate and high rates of ammonium sulfate applied at planting; in fact, the only notable difference at 15 d was the reduced growth of plants which did not receive N at planting.

Since there was no difference between splitting N application in two or three parts, it may be advisable to apply two rather than three applications, because less labor is required. Treatment 3 (farmer option modified by Jacome and Blue, 1980) could be advisable in case of germination failure. Farmers who avoid investing scarce, costly fertilizer wait until plants germinate. However, plants in this treatment were less vigorous at 15 d; this could make

plants more susceptible to pests and diseases, water stress and to be less competitive with weeds. Less root growth could mean a lower capacity for the roots to take up needed water and nutrients. Treatment 3 could be more risky than treatment 2 or 4. Since these results showed the need for N in the first 15 d, it may be advisable to apply part of the N at planting. More research is needed to determine if salt toxicity not obvious here may occur in other soils and under other climatic conditions before definite conclusions are possible. Several practices could be tested. One of them will be to add the fertilizer by leaving a greater distance between seeds and fertilizer. Another possibility is to use another source of N at planting, then use ammonium sulfate for the second application.

CHAPTER 11
SUMMARY AND CONCLUSIONS

A series of experiments was conducted in the glasshouse and field in order to identify soil factors limiting bean and maize production in the area surrounding Zamorano, Honduras, Central America. Due to the potential of bean inoculation in the region, more emphasis was given to the effect of these factors on bean growth, BNF, and yield.

Chemical analyses of the soils studied indicated adequate amounts of most of the nutrients except N and P, which are commonly deficient in tropical soils. Soil pH ranged from 5.1 to 5.6. These soils contained medium amounts of organic matter and had different management histories.

Bean

Pot experiments were conducted to study the performance of *Rhizobium* strains collected from Zamorano soils and introduced *Rhizobium* strains (CIAT from Colombia, and

Nitragin from Wisconsin). While on average, native strains produced results equivalent to the introduced strains, strain effects were cultivar and site specific. A mixture of native and introduced Rhizobium strains was used for subsequent studies. Despite good nodulation produced by inoculation, these treatments produced inferior results relative to those obtained with combined N. None of the combinations of corrective measures (reported below) were capable of making BNF as effective as use of combined N.

Low soil pH is common in tropical soils. This low pH is accompanied by deficiencies such as P, Ca and Mo, and some toxicities including Al, Mn and Fe. Because soils studied were not strongly acidic, a dramatic lime effect on plants was not expected.

The effect of pH on bean growth and BNF was studied in pot experiments by applying increasing levels of lime. In experiments with potted plants, application of up to two times the amount of lime needed to increase pH to 6.5 showed mixed results. In 1982, a linear response of plant dry weight to lime was obtained, followed by no response in one experiment. In 1983, no response was observed in either soil. However, in 1984, using similar soils, a quadratic negative response in one soil and quadratic positive response in the other soil were obtained when plant dry weight was measured. This lack of consistency is cited in the literature, and recommendations based only on pot

experiments should be avoided. In field experiments lime at the rate of 1.6 Mg $Ca(OH)_2$ ha^{-1} improved BNF and yield in both sites.

Phosphorus at the rates of 0, 20, 40, and 80 kg ha^{-1} was studied in pot experiments. In the first year, no response to P was observed. Lime was applied to increase pH to 7.0 in both soils. However, in the second year in unlimed soils, a linear response of bean dry weight was obtained even when the highest level of P applied was equivalent to seven times the amount normally recommended for beans in Central America (26 kg P_2O_5 ha^{-1}). In field experiments, response to P was positive in all experiments but differed among cultivars. Porrillo S. cultivar did not respond to P application in Horticultura even when extractable P was interpreted as being deficient. Existing recommendations appear to underestimate the P needed for beans. Since P fertilizers are very expensive, in Third World countries, and beans are not a cash crop, recommendations should consider economic and social realities.

Molybdenum levels ranging from 0 to 1 kg ha^{-1} were studied. The highest level of Mo studied was based on NifTAL (FAO, 1980) recommendations. A concern that the amount of Mo recommended by NifTAL could be toxic for Rhizobium strains was considered. In 1982 and 1983, no response to Mo was obtained. In 1984, the application of Mo with lime produced mixed results. In the limed Colindres soil, response of

Porrillo S. plants to Mo application was negative. The opposite occurred in the limed soil from Horticultura. Acacias 4 cultivar did not responded to Mo applications either with or without lime. Due to these inconsistent results, conclusions or recommendations on Mo fertilization can not be drawn from these experiments. There are at least three possible reasons for these variable results. Firstable, could be that Mo content in bean seeds varied among cultivars. Secondly, soil available Mo could differ. Third, could be that there are differences in the host plants in the ability to take Mo from the soil.

Applications of combined N were generally superior to inoculation, and positively interacted with the other factors studied (P, lime, and Mo). Plant dry weight, N in tissue, and yield were all increased by application of combined N as compared to inoculation. This supports the claim that even under favorable conditions, symbiotic N_2 fixation may provide only a small fraction of the bean plant's N requirement. On the other hand, the organic matter in the soils studied might provide part of the N required.

Irrigation treatments consisted of water applications every 2 d applying low, medium, and high levels of water (100, 200, and 300 L per plot, respectively). These rates estimated to be roughly 1/3, 2/3, and full field capacity, were imposed on the bean crop on two planting dates to determine the effect of water on bean growth, BNF, and

yield. This experiment was planted during the dry season that coincides with the hottest months. Water proved to be an important factor in the determination of plant dry weight, nodule weight, and yield. Bean yields from the best irrigation treatment in this study were equivalent to the yields obtained when combined N was applied in the other field experiments. This means that water plays a key role in improving bean growth as well as BNF and yield.

Upon the completion of this study, several important facts became evident which should be considered to improve bean production in Honduras. 1) Inoculation did not appear to be a viable option to combined N in these studies; response to inoculation was very inconsistent, and never better than that obtained with combined N. 2) Zamorano soils do not fall into the "extremely weathered" category, though they do lack P. Extensive research of this element should be conducted in order to determine the critical level on other Honduran soils. Responses of bean to applications of lime and Mo indicate that the need for these two amendments are not priorities. 3) Water is a very important factor in bean production. Ways to improve soil moisture conservation should be studied. These could include use of irrigation when possible and use of mulches such as maize stalks, rice hulls, or other readily available sources to the scarce-resource farmer.

Maize

The effect of different timings of split applications of ammonium sulfate on maize growth and yield was determined. The recommendation of CENTA/MAG (El Salvador) was compared to the modified farmer's technology of Jacome and Blue (1980) and two other treatments. The application of ammonium sulfate in three parts did not produce an advantage over treatments where this fertilizer was divided into two parts. The treatment suggested by Jacome and Blue (1980) resulted in lower plant dry weight, root dry weight and plant height at 15 d. However, no differences in maize grain yields were obtained. Delaying the application of ammonium sulfate for 15 d could be risky, since plants were smaller, and hence more vulnerable to pests and at the same time less able to obtain water and nutrients due to underdeveloped roots. No salt toxicity was observed. On the other hand, delayed fertilization could be advantageous in the case of low germination due to poor seed quality, lack of rain, and pest attacks. In this case, farmers will not have spend their limited resources on fertilizer. The application of N at planting could also cause salt toxicity if insufficient moisture is present to dissolve and disperse the N salts.

APPENDIX A
NUTRIENT SOLUTION USED FOR THE GLASSHOUSE EXPERIMENTS

Quantities of elements and reagents used to make a nutrient solution for glasshouse experiments in CATIE, Costa Rica (Diaz-Romeu and Hunter, 1978). This basic solution was modified according to objectives of the specific experiment.

Element	Reagent	Amount of reagent	Amount of nutrient applied
		$g\ L^{-1}$	$mg\ kg^{-1}$
N	NH_4NO_3	14.29	50
P	Conc. H_3PO_4	14.88	40
K	KCl	14.90	78
Mn	$MnCl_2.4H_2$	10.81	30
Cu	$CuCl_2.2H_2O$	0.54	2
Zn	$ZnCl_2$	2.08	10
S	Conc.H_2SO_4	9.50	30
B	H_3BO_3	1.15	2
Mo	$(NH_4)_6Mo_4O_{24}.4H_2O$	0.09	0.5
Fe	$FeCl_3.6H_2O$	9.68	20

APPENDIX B
MONTLY RAINFALL DISTRIBUTION FOR 1982 TO 1984 IN THE YEGUARE VALLEY, HONDURAS

Month	Year		
	1982	1983	1984
	----------------mm	---------------	
January	7.1	2.8	15.1
February	10.7	12.8	2.6
March	4.1	7.8	5.3
April	1.1	26.1	10.4
May	-- †	44.1	156.0
June	222.7	145.0	217.6
July	103.4	165.1	171.1
August	72.1	177.8	226.9
September	187.2	237.9	230.4
October	101.4	78.6	162.8
November	22.8	70.0	15.5
December	8.6	20.1	15.5
Total	741.2	988.1	1229.2

† Missing data

APPENDIX C
DAILY RAINFALL DISTRIBUTION FOR 1983 IN THE YEGUARE VALLEY, HONDURAS.

Day	Month											
	J.	F.	M.	A.	M.	J.	J.	A.	S.	O.	N	.D.
	mm											
1	0.0	0.0	0.0	0.0	0.0	0.2	0.3	0.0	28.2	5.3	0.0	1.1
2	0.0	0.0	0.0	-- †	0.0	9.7	17.6	0.1	0.0	11.5	23.5	1.9
3	0.6	0.0	--	0.5	0.0	5.5	8.5	7.2	0.4	3.9	3.4	3.3
4	0.0	0.0	0.0	0.0	0.0	0.3	0.2	--	1.3	0.5	12.4	0.0
5	0.0	0.0	0.0	0.0	7.1	0.0	2.9	3.7	0.4	20.5	0.0	2.6
6	0.0	0.0	0.0	0.1	0.0	0.0	0.1	3.7	7.6	5.5	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	17.7	15.6	0.1	11.8	0.0	7.3
8	0.0	0.0	0.0	0.0	0.0	1.8	24.6	14.6	4.3	0.3	9.7	0.0
9	0.0	0.0	0.0	0.0	3.8	25.1	0.0	36.6	2.3	0.0	0.0	0.0
10	0.0	0.0	5.1	--	0.1	17.7	0.0	0.3	11.5	0.0	0.5	0.0
11	0.0	0.0	0.0	0.0	0.0	0.2	1.7	3.0	13.4	0.0	--	0.0
12	2.0	0.0	0.0	0.0	0.0	4.9	2.0	--	2.3	1.4	5.1	0.0
13	0.0	9.1	0.0	0.0	0.0	1.9	0.8	0.0	--	0.3	4.6	0.0
14	0.0	0.0	0.0	0.0	0.0	26.0	11.2	0.0	0.0	1.3	--	0.0
15	0.0	0.0	0.0	5.2	0.0	1.3	0.1	0.0	0.0	0.0	0.0	0.9
16	0.0	0.1	2.3	0.7	0.0	--	2.5	0.2	6.6	0.0	--	0.0
17	0.0	0.0	0.0	0.0	0.0	3.1	23.6	0.0	1.8	0.1	--	0.3
18	0.0	0.0	0.0	0.0	0.0	3.5	0.7	0.0	4.1	0.5	0.0	0.3
19	0.0	0.0	0.0	0.0	0.0	23.2	0.0	19.1	4.8	0.2	0.0	0.3
20	0.0	0.0	0.0	0.0	0.0	--	--	17.8	3.2	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	14.5	0.0	4.4	--	0.0	0.0	0.0
22	0.0	0.0	0.0	0.1	0.0	0.0	24.6	11.2	0.0	0.0	5.6	0.0
23	0.0	0.0	0.0	0.0	0.0	0.0	0.3	2.4	0.0	2.2	0.0	2.1
24	0.0	--	0.0	0.0	0.0	0.0	18.5	2.1	--	2.5	0.0	0.0
25	0.0	3.2	0.4	19.7	0.0	0.5	0.2	0.7	5.6	0.0	0.0	0.0
26	0.0	--	0.0	0.0	0.0	--	0.1	0.0	31.0	8.2	0.5	0.0
27	0.0	0.0	0.0	0.0	0.0	1.1	4.6	5.0	1.7	2.6	0.0	0.0
28	0.0	0.4	0.0	0.0	0.0	0.0	1.1	5.0	8.7	0.0	0.0	0.0
29	0.0		0.0	0.0	0.0	0.6	1.2	3.3	20.8	--	0.2	0.0
30	0.0		0.0	0.0	0.2	4.9	0.0	2.3	77.8	0.0	4.5	0.0
31	0.2		0.0		32.9		0.0	19.5		0.0		0.0
T	2.8	12.8	7.8	26	44	145	165	178	237.9	78.6	70	20

† Missing data

APPENDIX D
DAILY RAINFALL DISTRIBUTION FOR 1984 IN THE YEGUARE VALLEY, HONDURAS.

Day	Month											
	J.	F.	M.	A.	M.	J.	J.	A.	S.	O.	N.	D.
	mm											
1	0.0	0.0	0.0	0.0	0.0	0.0	3.5	20.1	19.2	5.6	4.9	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	5.4	12.2	14.3	0.1	0.1	0.9
3	0.0	0.3	0.0	0.0	0.0	50.5	2.2	18.1	11.4	7.8	0.0	2.2
4	0.0	0.0	0.0	0.0	0.0	7.2	0.8	0.5	3.4	11.3	0.0	0.2
5	0.0	0.0	0.0	0.0	4.5	24.3	6.2	--	6.3	2.6	2.6	--
6	0.0	0.1	0.0	0.0	0.0	0.0	6.6	4.2	1.5	6.3	0.0	0.0
7	0.0	0.0	0.4	0.2	0.0	0.0	3.6	--	--	7.8	0.0	0.0
8	0.0	0.0	--	0.0	0.0	0.0	19.1	0.0	2.9	0.0	0.0	0.0
9	0.0	0.0	--	0.0	0.0	18.0	17.1	0.0	18.7	2.9	0.0	0.0
10	0.0	0.0	--	0.0	0.0	0.5	0.3	0.0	8.4	1.1	0.0	0.0
11	0.0	0.0	0.0	0.0	0.0	7.6	0.0	0.2	5.0	0.0	4.1	0.0
12	0.0	0.0	0.0	0.0	0.0	21.2	0.0	30.3	4.1	0.0	--	0.2
13	0.0	0.0	0.0	0.0	0.0	2.8	18.5	14.8	0.2	0.0	0.0	0.0
14	0.6	1.8	0.0	0.0	1.8	32.6	--	0.0	--	0.0	0.0	0.0
15	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	5.9	0.0	2.6
16	2.8	0.0	0.0	0.0	0.0	1.1	0.0	0.6	32.2	13.5	0.0	0.1
17	0.9	0.0	0.0	0.0	0.0	9.5	7.5	0.0	18.0	0.0	0.1	0.0
18	0.0	0.0	4.5	0.0	0.0	0.9	5.7	16.5	1.8	1.4	1.7	0.0
19	2.8	0.0	0.0	0.0	0.0	0.0	12.5	4.0	--	0.0	1.2	0.0
20	2.8	0.0	0.0	0.0	0.0	21.4	0.2	0.5	0.0	0.0	0.8	0.0
21	0.8	0.0	0.0	0.0	0.0	0.0	0.5	42.0	--	0.0	--	0.0
22	--	0.3	0.0	0.0	0.0	0.0	0.3	0.1	1.3	17.2	0.0	0.0
23	4.4	0.0	0.0	0.0	0.1	0.0	24.5	0.0	7.3	0.2	0.0	0.0
24	0.0	0.0	0.0	0.1	0.0	9.0	9.2	0.0	15.3	0.5	0.0	0.0
25	0.0	0.0	0.4	10.1	0.0	1.1	2.8	7.0	33.7	0.0	0.0	0.0
26	0.0	0.0	0.0	0.0	9.7	0.0	10.0	2.9	0.1	--	0.0	0.0
27	0.0	0.0	0.0	0.0	5.4	2.6	1.6	1.0	8.2	8.4	0.0	3.7
28	0.0	0.1	0.0	0.0	1.6	--	0.5	--	17.0	0.0	0.0	2.8
29	0.0	0.0	0.0	0.0	14.8	6.4	5.3	11.7	0.1	58.0	0.0	1.1
30	0.0		0.0	0.0	15.4	0.8	2.1	0.3	0.0	12.0	0.0	0.4
31	0.0		0.0		102.7		5.3	39.9		0.2	0.0	1.3
T	15	2.6	5.3	10.4	156.0	217.6	171	226.9	230	162.8	15.5	16

† Missing data

APPENDIX E
CONCENTRATION OF NUTRIENTS IN BEAN PLANTS
ACCORDING TO HOWELER (1983)

Nutrient	Deficient	Low	Optimum	High	Toxic
N (%)	<3	3-4.5	4.5-5.5	>5.5	----
P (%)	<0.25	0.25-0.35	0.35-0.50	>0.5	----
K (%)	<1	1-2	2-4	>4	----
Ca(%)	<1.25	1.25-1.3	1.3-2.0	>2	----
Mg(%)	<0.3	0.3-0.35	0.35-1.3	>1.3	----
S (%)	<0.14	0.14-0.20	0.2-0.3	>0.3	----
B (ppm)	<15	15-20	20-30	30-40	>45
Cu (ppm)	<15	----	15-25	----	----
Fe (ppm)	<100	----	100-800	----	----
Mn (ppm)	<20	20-80	80-200	----	>200
Zn (ppm)	<15	15-40	40-50	----	----

APPENDIX F
NUTRIENT CONTENT OF SOIL SAMPLES FROM THE LIME EXPERIMENT FOR THE HORTICULTULTURA SOIL IN HONDURAS, 1984.

Lime [½$Ca(OH)_2$]	Mehlich I-extractable soil nutrient P	Ca	Mg	K	Cu	Fe	Zn	Mn
cmol kg^{-1}	---------------mg kg^{-1}							--------------
0	43	1950	124	314	0.9	13	12	53
0.9	31	1975	138	242	1.0	11	9	61
1.9	34	2235	131	247	0.9	15	6	60
2.9	31	2350	136	302	0.8	14	8	63
3.8	30	2320	133	327	0.7	10	10	57

APPENDIX G
CORRELATION COEFFICIENTS FOR SOIL VARIABLES FOR THE LIME EXPERIMENT IN HONDURAS IN 1984.

	Cu	Ca	Mg	K	Fe	Mn	Zn	P
Cu	1.00							
Ca	0.72	1.00						
Mg	0.15	-0.13	1.00					
K	0.20	0.04	-0.15	1.00				
Fe	0.07	0.15	-0.08	0.19	1.00			
Mn	0.03	-0.19	0.91	-0.20	-0.35	1.00		
Zn	0.26	-0.05	0.20	-0.10	-0.32	0.22	1.00	
P	0.20	0.03	0.40	-0.35	0.07	0.34	-0.16	1.00

APPENDIX H
NUTRIENT CONTENT OF FOLIAR SAMPLES FROM THE LIME EXPERIMENT FOR THE COLINDRES SOIL IN HONDURAS, 1984.

Lime [½$Ca(OH)_2$]	Oven-dry bean plant nutrients						
	P	Ca	Mg	Cu	Fe	Zn	Mn
cmol kg^{-1}	----g kg^{-1}----			-----mg kg^{-1}------			
			Acacias 4 cv.				
0	2.0	12.8	2.0	10	124	56	115
0.9	2.7	13.0	2.3	6	223	41	111
1.9	2.3	13.6	2.1	6	170	37	87
2.8	2.6	14.6	2.4	7	233	38	91
3.7	2.2	15.6	2.3	7	120	46	81
			Porrillo S. cv.				
0	2.4	13.5	2.3	10	159	50	156
0.9	2.3	14.3	2.1	5	126	39	93
1.9	2.3	16.4	2.4	6	130	33	110
2.8	2.6	16.1	2.4	7	136	39	88
3.7	2.3	16.0	2.1	6	130	42	71

APPENDIX I
CORRELATION COEFFICIENTS FOR PLANT VARIABLES FOR THE LIME EXPERIMENT IN HONDURAS IN 1984.

	Cu	Ca	Mg	Fe	Mn	Zn	Mo	Al	P
Cu	1.00								
Ca	0.15	1.00							
Mg	-0.13	0.46	1.00						
Fe	0.66	0.19	0.46	1.00					
Mn	0.35	0.70	0.57	0.57	1.00				
Zn	0.17	0.42	0.54	0.50	0.78	1.00			
Mo	0.07	-0.69	-0.49	-0.11	-0.57	-0.43	1.00		
Al	-0.01	0.26	0.10	0.25	0.34	0.16	-0.22	1.00	
P	-0.07	0.02	0.11	-0.06	-0.26	-0.21	-0.04	-0.34	1.00

APPENDIX J

NUTRIENT CONTENT OF SOIL SAMPLES FROM THE P EXPERIMENT FOR THE COLINDRES SOIL IN HONDURAS, 1984.

P applied	Mehlich I-extractable soil nutrients							
	P	Ca	Mg	K	Cu	Fe	Zn	Mn
$mg\ kg^{-1}$	$mg\ kg^{-1}$							
	Acacias 4 cv.							
0	5.9	1350	119	233	1.8	22	3.5	42
10	7.0	1195	122	221	2.2	21	3.5	38
20	9.7	1230	126	221	2.0	22	4.0	43
40	9.3	2170	140	260	1.9	13	9.2	47
	Porrillo S. cv.							
0	5.4	1620	116	231	1.6	19	3.7	42
10	7.2	1560	128	241	1.8	20	3.8	43
20	9.8	1795	125	240	1.6	16	4.1	37
40	16.2	1465	116	236	1.8	18	3.4	42

APPENDIX K
NUTRIENT CONTENT OF SOIL SAMPLES FROM THE P EXPERIMENT FOR THE HORTICULTURA SOIL IN HONDURAS, 1984.

P applied	Mehlich I-extractable soil nutrients							
	P	Ca	Mg	K	Cu	Fe	Zn	Mn
$mg\ kg^{-1}$	---------------$mg\ kg^{-1}$---------------							
				Acacias 4 cv.				
0	24.7	2550	157	293	0.7	13	11.0	42
10	30.5	1380	115	248	1.0	17	5.7	47
20	30.5	2000	134	256	0.9	14	7.5	41
40	31.3	1475	126	230	0.8	15	6.1	40
				Porrillo S. cv.				
0	22.1	2020	133	274	1.1	15	12.3	50
10	29.2	2375	142	275	0.8	11	12.0	39
20	32.3	2080	129	239	0.9	17	12.0	40
40	30.4	2275	120	275	0.9	10	9.5	47

APPENDIX L
CORRELATION COEFFICIENTS FOR SOIL VARIABLES FOR THE P EXPERIMENT IN HONDURAS IN 1984.

	Cu	Ca	Mg	K	Fe	Mn	Zn	P
Cu	1.00							
Ca	-0.56	1.00						
Mg	-0.25	0.56	1.00					
K	-0.57	0.81	0.60	1.00				
Fe	0.66	-0.80	-0.39	-0.67	1.00			
Mn	-0.02	0.15	-0.01	0.22	-0.03	1.00		
Zn	-0.65	0.79	0.51	0.71	-0.69	0.28	1.00	
P	-0.85	0.45	0.15	0.42	-0.63	0.07	0.64	1.00

APPENDIX M
NUTRIENT CONTENT OF FOLIAR SAMPLES FROM THE P EXPERIMENT FOR THE TWO SOILS IN HONDURAS, 1984.

Cultivar	P applied	Oven-dry bean plant nutrient concentration						
		P	Ca	Mg	Cu	Fe	Zn	Mn
		-----mg kg^{-1}-----			-----mg kg^{-1}-----			
		Colindres soil						
Acacias	0	1.6	13.9	2.0	6.5	150	40	82.5
	10	1.6	12.6	1.8	9.3	225	34	81.3
	20	1.5	12.8	2.0	7.0	160	51	76.3
	40	1.9	11.9	1.8	8.4	136	39	68.8
		Horticultura soil						
Porrillo	0	1.5	13.4	1.9	8.2	151	65	81.3
	10	2.2	14.8	2.3	6.3	160	44	72.5
	20	2.1	13.7	2.2	8.1	166	47	80.0
	40	2.1	13.3	2.2	6.1	223	49	86.3

APPENDIX N
CORRELATION COEFFICIENTS FOR PLANT VARIABLES FOR THE P EXPERIMENT IN HONDURAS IN 1984.

	Cu	Ca	Mg	Fe	Mn	Zn	Mo	Al	P
Cu	1.00								
Ca	0.09	1.00							
Mg	-0.30	0.40	1.00						
Fe	-0.24	0.13	0.47	1.00					
Mn	-0.17	0.08	0.50	0.29	1.00				
Zn	0.31	0.19	0.18	0.03	0.05	1.00			
Mo	0.01	0.25	0.03	-0.08	-0.36	-0.02	1.00		
Al	-0.09	0.35	0.37	0.50	0.15	0.41	-0.09	1.00	
P	-0.15	0.17	0.60	0.38	0.33	0.06	0.18	0.14	1.00

APPENDIX O
NUTRIENT CONTENT OF SOIL SAMPLES FROM THE Mo EXPERIMENT IN COLINDRES SOIL, IN HONDURAS, 1984.

Lime [½Ca(OH)$_2$]	Mo	Mehlich I-extractable soil nutrients						
		Cu	Ca	Mg	K	Fe	Zn	Mn
cmol kg^{-1}	mg kg^{-1}	---------------mg kg^{-1} ----------------						
		Acacias 4 cv.						
0	0	0.9	885	106	172	2.8	4.0	48
	0.06	1.7	900	105	172	2.7	4.4	52
	0.13	1.9	965	108	197	3.0	4.1	58
	0.25	0.9	870	102	171	3.1	4.2	49
	0.50	2.2	910	105	181	2.7	3.5	47
1.9	0	0.6	1440	120	191	1.7	5.2	35
	0.06	1.8	1175	114	178	1.0	3.3	34
	0.13	2.1	1495	128	179	4.9	3.9	41
	0.25	2.1	1360	119	191	5.9	3.8	34
	0.50	0.4	1325	114	168	1.7	3.9	29
		Porrillo S. cv.						
0	0	1.1	1005	120	177	2.6	4.3	46
	0.06	1.0	845	98	161	2.7	3.9	38
	0.13	0.9	780	95	148	2.8	3.9	40
	0.25	1.0	830	99	157	3.2	3.6	44
	0.50	0.9	780	99	164	3.4	3.9	42
1.9	0	2.0	1600	140	214	4.8	4.1	42
	0.06	0.4	1330	108	167	1.8	4.3	26
	0.13	0.9	1253	108	141	2.4	3.9	29
	0.25	1.7	1355	109	174	3.3	5.1	32
	0.50	0.7	1390	114	179	2.3	3.7	29

APPENDIX P

NUTRIENT CONTENT OF SOIL SAMPLES FROM THE Mo EXPERIMENT IN HORTICULTURA SOIL, IN HONDURAS, 1984.

Lime [½$Ca(OH)_2$]	Mo	Mehlich I-extractable soil nutrients						
		Cu	Ca	Mg	K	Fe	Zn	Mn
cmol kg^{-1}	mg kg^{-1}	mg kg^{-1}						
		Acacias 4 cv.						
0	0	0.6	1140	107	145	2.5	3.6	36
	0.06	2.5	930	107	185	1.9	3.9	52
	0.13	0.6	1167	104	153	2.9	4.3	40
	0.25	1.0	755	89	151	4.0	3.6	55
	0.50	1.0	2065	142	267	8.4	9.6	49
1.9	0	1.1	1950	144	265	8.6	10.0	43
	0.06	0.9	1870	143	272	4.8	11.0	45
	0.13	0.8	1980	150	278	5.0	12.0	42
	0.25	1.1	2250	154	292	7.6	12.5	42
	0.50	1.0	1285	114	231	3.9	8.2	36
		Porrillo S. cv.						
0	0	1.2	1753	141	262	8.9	9.5	52
	0.06	0.3	1370	121	231	4.5	9.9	41
	0.13	0.4	1460	126	229	7.6	9.4	41
	0.25	0.4	1525	137	225	4.9	10.3	43
	0.50	0.3	1695	132	227	4.1	10.0	36
1.9	0	0.8	1830	135	264	4.6	9.3	43
	0.06	0.6	1700	123	241	5.3	9.7	42
	0.13	0.3	1700	133	224	2.9	10.0	27
	0.25	0.3	1715	130	235	2.0	8.9	36
	0.50	0.9	1675	139	275	5.5	8.7	46

APPENDIX Q
CORRELATION COEFFICIENTS FOR SOIL VARIABLES FOR THE Mo EXPERIMENT IN HONDURAS IN 1984.

	Cu	Ca	Mg	K	Fe	Mn	Zn
Cu	1.00						
Ca	-0.20	1.00					
Mg	0.11	0.78	1.00				
K	-0.28	0.86	0.78	1.00			
Fe	0.77	0.21	0.43	0.16	1.00		
Mn	-0.23	0.23	0.35	0.48	0.05	1.00	
Zn	-0.33	0.70	0.60	0.80	0.54	0.10	1.00

APPENDIX R
NUTRIENT CONTENT OF FOLIAR SAMPLES FROM THE Mo EXPERIMENT FOR THE TWO SOILS IN HONDURAS, 1984.

Lime	Oven-dry bean plant nutrient concentration								
[½Ca(OH)$_2$]	P	Ca	Mg	K	Cu	Fe	Zn	Mn	Mo
cmol kg^{-1}	-------g kg^{-1}-------				--------mg kg^{-1}--------				
				Colindres soil					
0	2.1	13.7	19.9	28.9	7.1	158	42	78	3.9
1.9	1.9	13.9	19.8	28.5	6.5	154	43	110	2.0
				Horticultura soil					
0	1.9	13.2	21.4	26.7	6.2	165	40	133	0
1.9	2.2	13.6	23.1	30.5	6.8	187	48	134	0

APPENDIX S
CORRELATION COEFFICIENTS FOR PLANT VARIABLES FOR THE Mo EXPERIMENT IN HONDURAS IN 1984.

	Cu	Ca	Mg	K	Fe	Mn	Zn
Cu	1.00						
Ca	0.09	1.00					
Mg	0.05	0.23	1.00				
K	0.25	0.36	0.33	1.00			
Fe	0.17	0.10	0.30	0.25	1.00		
Mn	-0.08	-0.05	0.45	-0.01	0.39	1.00	
Zn	0.38	0.20	0.18	0.22	0.22	0.12	1.00

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BIOGRAPHICAL SKETCH

The author was born in El Salvador, Central America, daughter of Julio Chavez and Dolores de Chavez. She received her Bachelor of Science degree in agricultural engineering at the National University of El Salvador in 1975 and worked for several years with the National Center of Agricultural Technology of the Ministry of Agriculture of El Salvador (CENTA/MAG), doing soil analyses and working in glasshouse research with sorghum and sunflowers. At the end of 1975, the Organization of American States, together with the Belgium government, offered her one of the five Latin American scholarships to study for her master's degree in soil science at the International Centre for Graduate Soil Scientists, State University of Ghent. After returning to her country in 1977, the author worked as a researcher in fertilization of rice, cassava, green peppers, and beans. During that time she worked as a counterpart of the soil scientist, Dr. Frank Calhoun, from University of Florida in the classification of Salvadoran soils. The author worked as a subdirector of the Research Division. The author began to be interested in Rhizobium and the potential practical use

of inoculation of beans in the area and started working for her Ph.D. program with Drs. J. B. Sartain and D. Hubbell at the University of Florida through a USAID/CSRS grant, doing her research in Honduras. In Honduras, the author taught at the Escuela Agricola Panamericana and installed the soil microbiology laboratory.

The author is married to Keith L. Andrews. They have three children, Jose Eduardo 17, Brian James 7, and Johanna Yvonne 5, who are currently living in Honduras, Central America.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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